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SOME STUDIES ON BORDEAUX MIXTURE

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I. SUMMARY

1. When dilute solutions and weaker mixtures are used a material saving is made.

2. Thorough and violent stirring so breaks up the colloidal membranes formed in the mixture that they do not settle rapidly; but it will not entirely prevent settling.

3. Sphere crystals are formed in a short time from the copper salts in all of the ordinary mixtures. The covering power and adhesiveness of these bodies is very much less than that of the colloidal membranes.

4. Bordeaux mixture may be kept indefinitely, physically and chemically as when first made, by the addition either of a little cane sugar or of glucose.

5. Bordeaux mixture is fungicidal immediately upon its application.

6. The covering of a sprayed leaf consists of thin colloidal membranes composed of copper salts and of particles of lime.

7. The copper in the thinner membranes is immediately soluble and fungicidal; that in the portions adjacent to the lime particles gradually becomes soluble.

8. The lime particles are fungicidal.

9. When spores fall on a moistened leaf sprayed with bordeaux they are either: (a) killed, (b) their germination is retarded, or (c) they put forth a short germ-tube which is not likely to grow.

10. Bordeaux mixture kills largely by contact. The drip water is not highly fungicidal.

11. Bordeaux mixture under field conditions increases transpiration in the freshly sprayed plants.

12. Bordeaux mixture increases the size of the cells and of the chromatophores and nuclei of the sprayed leaves.

13. Bordeaux mixture increases the yield from potato plants by the prevention of tip-burn and flea beetle injury.

14. When plants which are not troubled by tip-burn or flea beetle are sprayed, yields are not increased.

15. At least two-thirds of the injury to late potato varieties other than that wrought by fungus or bacterial attack is due to tip-burn. The residue of the injury is due to the flea beetle.

16. In the greenhouse or in climates where neither tip-burn nor flea beetle are a factor in potato growing, or where neither early nor late blights occur, bordeaux mixture seems in the long run to be neither beneficial nor harmful, but quite unnecessary.

II. INTRODUCTION

The following studies on bordeaux mixture in its fungicidal and physiological relations are preceded by a brief statement of its chemical and physical properties, since a clear understanding of these facts is necessary if correct conclusions are to be drawn as to the changes induced in fungi or on the sprayed plants. Many students of bordeaux usage have disregarded the physical properties entirely, an omission so fatal that it is not too much to say that their work has no solid foundation. The writer does not claim to understand the chemistry of the mixture as it is ordinarly made, but in this respect he is not in a much worse condition than the chemists themselves. The entire physiological problem is a complex one and, as will be pointed out, it is not the same in different climates. However, the conclusions that are presented here seem to be true for Vermont.

III. PHYSICAL AND CHEMICAL PROPERTIES

The chemical nature of the compound or compounds formed when solutions of copper sulphate and calcium hydroxid are brought together is so complex and varying that it is possible only for a chemist to discuss the matter properly. The earlier view that only one compound, copper hydroxid, was formed and that this was the principal fungicidal constituent, has been replaced by the later one that as a result of the reaction, a basic copper sulphate, and probably a series of basic copper sulphates, is precipitated. Pickering (34), who has studied this series carefully, has found that different substances are formed if the ratio of the lime and copper sulphate solutions is changed. He used apparently only combinations of copper sulphate solutions and clear lime water to obtain six basic sulphates, enumerated by him as follows:—

"A. 4 CuO, SO_3 (or 10 CuO, 2.5 SO_3).

B. 5 CuO, SO_3 (or 10 CuO, 2 SO_3).

C. 10 CuO, SO_3.

D. 10 CuO, SO_3, CaO.

E. CuO, 2 CaO (or 10 CuO, 20 CaO) existence doubtful.

F. CuO, 3 CaO (or 10 CuO, 30 CaO)."

If he succeeded in doing anything with the chemical analysis of the ordinary mixture made with milk of lime, undoubtedly he would have found many more. The fact that he does not give such an an-

alysis is evidence of the difficulties in the way of determining resultants. The only thing that can be said is that the combinations must be numerous, very complex and probably varying in composition. The compound that is formed is of a colloidal nature, membranous in character, and apparently is not much heavier than the liquid in which it floats. The shape of these membranes, when isolated and dried to a slide, is shown in figure 1. These colloidal membranes, whatever their chemical composition, be it simple or complex, formed chemically in the matter above indicated, constitute the essential and valuable feature of the mixture. They float suspended in a strong solution of calcium hydroxid and calcium sulphate, the only purpose of which seems to be to hold them apart and to dilute the suspension.

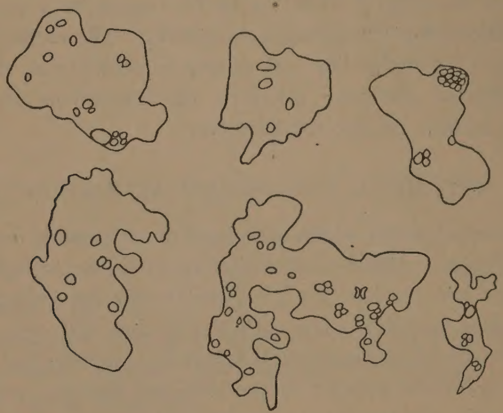


FIG. 1. Form of bordeaux membranes, showing particles of lime within.

The area of these membranes is a measure of the actual covering power of the mixture, as the author has shown in a previous article (29), and some combinations, such as that of Pickering's lime water and weak copper sulphate solution, the so-called Woburn bordeaux of England, while apparently containing a large amount of film do not in reality carry any considerable quantity. The method used in the measurement of these films was to dilute the mixture either 200 or 400 times; to spread half a cubic centimeter of this dilution on a glass slide and allow it to dry there; to remove the excess of lime and change the appearance of the copper by washing the slide with a weak solution of potassium ferricyanide acidified with a little acetic acid; to ascertain the area of the spot on the slide by means of a planimeter; to draw on paper a series of fields with the help of a camera lucida netz-micrometer

eye-piece; to determine the proportion of the area covered by the bordeaux films by measuring the area of the larger ones with a planimeter and by estimating that of the smaller; and, from the proportional amount covered by the film, the area of the half centimeter spot, and the dilution, to calculate the total amount of surface that the films produced in a certain mixture would cover. As far as it was possible, the conditions of preparation were made uniform throughout the trials. Several experimental mixtures were made and many samples measured in order to secure a representative average. The variable proportions of lime, especially under controlled conditions, and the difficulties experienced in pipetting so as to get a representative sample, are obvious.

The area covered by one cubic centimeter of the various mixtures tested was as follows:—

Pickering's bordeaux mixture (saturated lime-water) ..	70.6 sq. cm.
2½-2½-50 bordeaux mixture	111.8 sq. cm.
5-5-50 bordeaux mixture	257.0 sq. cm.
10-10-50 bordeaux mixture	393.6 sq. cm.
20-20-50 bordeaux mixture	498.8 sq. cm.
Lime 5, copper sulphate to neutrality	296.6 sq. cm.

As concentration increased so did the area of film become greater; but the increase was not proportional to that of the materials used. The inference to be drawn from this fact is that the colloidal films are thicker when more concentrated solutions are used, that they cannot be broken up as readily, thus wasting materials. On the other hand, the very dilute solutions, such as Pickering's, possess a greater covering power for the amount of materials used.

The particles of lime either left uncombined in the liquid or enclosed in the membranes play an important role in the fungicidal action of the mixture and also in the rapidity with which it settles. It is highly important that the precipitate should remain in suspension for as long a time as possible for the reason that often some hours elapse between the preparation of the mixture and its application. The changes in fungicidal properties induced by these particles will be discussed under the section on fungicidal action (page 15), but their relation to the rapid settling of the mixture properly may be discussed here.

The lime particles enclosed in the membranes add weight to the latter and cause them to sink more rapidly. The larger and heavier the weight inside the membrane the more rapidly it falls. Furthermore in its descent it carries with it or accelerates the fall of other and less heavily loaded membranes which may be in its way. The presence of lime particles not surrounded by this colloidal membrane may

be considered as doubtful, although they may occur. In some instances at least, the membrane is not much larger than is the lime particle and does not serve to buoy it up or appreciably to hinder its sinking. The path of such a lime fragment can be followed along the side of a glass vessel containing bordeaux. Not only does it fall rapidly but it also carries other lighter particles with it.

The effect of stirring the mixture on the rapidity of settling has been studied repeatedly. The most recent work on this point which has come to the writer's attention is that of Hawkins (21), who added dilute lime to dilute copper sulphate solution or vice versa and shook the resultant precipitate 5, 15, 25, or 35 times before placing the mixture in jars to measure the rate of its subsidence. He found that the way in which the components were mixed was not as important as was the subsequent stirring. No particular advantage was gained by pouring the two solutions together, as recommended by Galloway in 1896. In this connection it should be noted that the approved German method of combining the two is to pour the diluted copper sulphate solution into the diluted lime and to agitate the precipitate. Theoretically this is a correct procedure, for the films of copper sulphate then contain no lime particles to weight them down and send them to the bottom; but practically, all the copper sulphate solution is used up in the reaction and the films always enclose more or less lime water and lime particles. The Galloway method when carried out by hand is open to the objection that, as the lime and copper sulphate solutions are poured together over a strainer, the former always lags a few seconds behind the latter, and, as a consequence, there is always a heavy deposit of the larger bits of lime in the dregs which belatedly is added to the mixture after all the copper sulphate solution has been poured in and the reaction to a greater or less extent has taken place.

The stirring probably exerts several effects on the mixture, providing it is done vigorously.

1. The larger membranes containing considerable fragments of lime are broken open and the lime particles are released and sink while the lightened membranes remain in suspension.

2. The larger pieces of lime not enclosed within the membrane walls are kept from dragging down the lighter membranes.

3. The larger membranes are broken up, their surface area is increased, and thus their buoyancy is made greater per unit of weight.

If strong solutions of milk of lime and copper sulphate, such as occur in 10-10-50 or 20-20-50 bordeaux, are used, the resulting mem-

branes are too thick to be broken readily by any ordinary stirring. If, however, either of the solutions is used in a dilute form, the membranes are not resistant, as Hawkins (21) has shown, for the reason that subsequent vigorous agitation will break them up. Even the most rapid agitation will not serve the purpose if the two solutions are very concentrated, as is indicated by the results obtained at this Station as cited below.

1. Dilute solutions, such as are used for 5-5-50 bordeaux, were poured together in the usual way and stirred from 10 to 15 times.

2. Dilute solutions (5-5-50) were poured as usual, and the resulting mixture was beaten for five minutes with an ordinary rotary egg beater.

3. A dilute solution of copper sulphate was poured into a dilute solution of lime and stirred from 10 to 15 times.

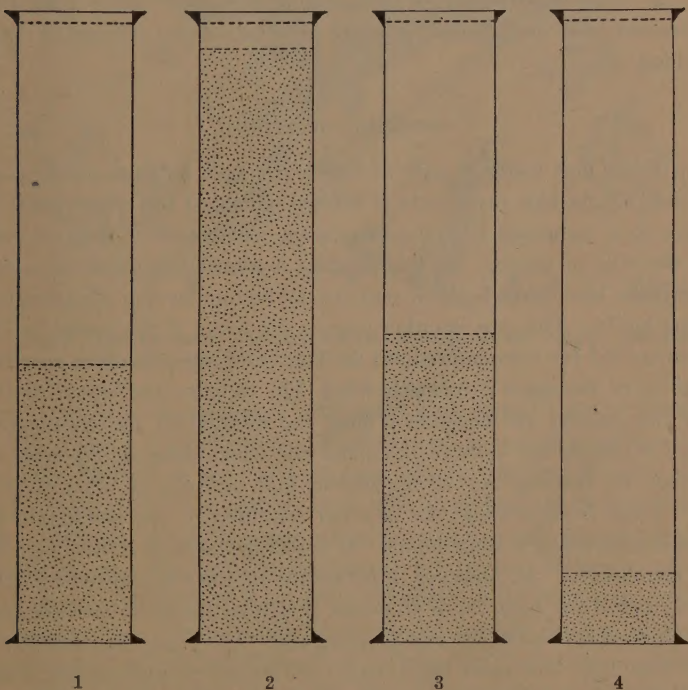


Fig. 2. Jars of bordeaux six hours after preparation. Previous treatment as follows: (1) 5-5-50 mixture stirred 10-15 times. (2) 5-5-50 mixture beaten for five minutes with an egg beater. (3) 5-5-50 solutions used, practically no stirring. (4) concentrated solutions, mixture beaten with egg beater.

4. Dilute copper sulphate and lime solutions were poured together but the mixture was stirred only enough to mix the two components.

5. Concentrated lime and copper sulphate solutions (one pound of either to the gallon) were poured together and the mixture beaten for five minutes with an egg beater.

The conditions relating to completeness of suspension after six hours are manifested in figure 2.

A study of the diagram clearly shows that even the violent stirring of the egg beater was not sufficient to make all the films buoyant. Practically all the precipitate in the dilute solution-egg beater trial was still in suspension after six hours, a remarkably satisfactory result.

Two facts of practical importance are to be deduced from these observations:—

1. The economy of using dilute materials.

2. The impossibility of so agitating a mixture made from concentrated lime and copper sulphate solutions as to prevent it from settling.

SETTLING AND AGING

When lime water or milk of lime and copper sulphate solution are mixed, a light blue precipitate is formed which, as has been shown on page 6, is composed of the precipitation membranes of one or more of the salts of copper. If this mixture is allowed to stand for a few hours the blue flakes begin to settle to the bottom leaving a supernatant clear liquid. This precipitation process continues if the vessel be left undisturbed for some hours until the blue solid occupies often less than a third of the space it formerly filled. The rapidity and extent of this settling process are dependent upon the dilution of the constituents used in making the mixture, the manner in which these were poured together, the thoroughness of the stirring and, perhaps, on other factors.

Aside from settling, the physical condition of the precipitate remains unaltered for some hours. It is still in the main a mass of films or membranes. If, however, a jar of the mixture is allowed to stand undisturbed for from 24 to 48 hours, it will be found to have changed its physical state radically and permanently. The precipitate still shows the same light blue color but, if examined under the microscope, it will be found that to a large extent the films have disappeared and that its hue is due to the presence of large numbers of blue spherical crystalline bodies (fig. 3, also plate I). Swingle (42) pointed out this fact, but its importance, indeed its very existence, does not seem to have be-

come appreciated generally, even by those who use bordeaux. Most bordeaux users throw away any mixture that has been left over night, believing apparently that it has spoiled chemically. Chemical changes may occur, but the important difference between a freshly made bordeaux and that which has stood for several days is physical in its char-

Fig. 3. Old bordeaux showing sphere crystals of the copper compound and long needle-like crystals of calcium sulphate.

acter; the copper compound or compounds have passed from a colloidal condition into a crystalline one. Furthermore, part of the calcium sulphate crystallizes in the form of long, slender needles that are frequently twinned. The precise form which calcium sulphate assumes when bordeaux is freshly made is unknown, but since it is only slightly soluble in water it must be in the form of a very fine precipitate, which either is amorphous or else too finely crystalline to be recognized under the microscope. The possibility remains also that the calcium sulphate is not formed at once but that it is the later product of some secondary reaction. At any rate it is present in an old bordeaux mixture in the form of the needle crystals. The excess of lime appears as small irregular granules.

The formation of these copper crystalline bodies in the precipitate evidently is due to an increased concentration of the copper salts at the

bottom of the vessel, as may be thus illustrated. If a hydrometer jar of bordeaux is left standing over night, the next morning it will be found usually that while the blue precipitation membranes on the surface of the blue layer have remained unchanged, there is an abundance of these copper spheres at the bottom. Intermediate stages in the

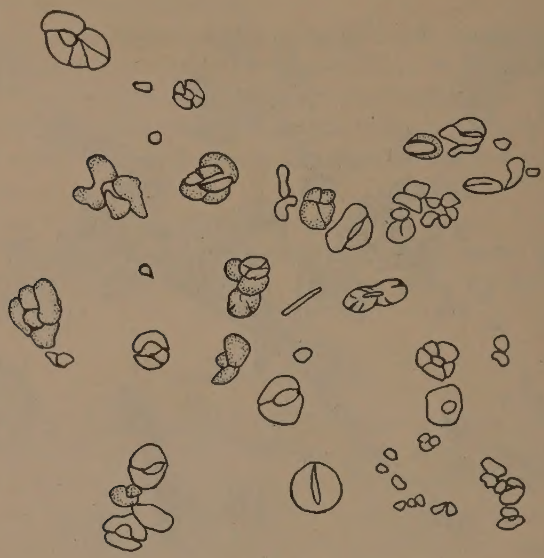


FIG. 4. Sphere crystals forming in old bordeaux; younger stages in their growth.

growth of these crystalline bodies may be found between the top and bottom of the precipitate. Although the earliest condition in which they can be recognized usually is to be seen while they are located within the old collodial membrane, this does not occur always since they often seem to lie free. The growth apparently begins as a rule around one of the bits of lime, as the crystals show a clean center with two or three blue projections when they are first seen. Then the later growth fills in and rounds out this irregular body, making it appear approximately spherical (fig. 4). If, however, instead of pouring the mixture into a cylinder to remain undisturbed, the same quantity is spread out in a thin layer of not over a half inch depth, as in a large Petri dish, these crystalline bodies are not produced. A saturated solution seems to be a prerequisite if a physical rearrangement of the copper molecules is to occur.

The ultramicroscope has revealed the structure of the colloids to be similar to that of crystalline substances. Hence this transition to a crystalline state is quite conceivable, much more so than it would have been in the earlier days when colloids were believed to possess a structure resembling that of a sponge.

Butler (7) has given considerable study to the formation of these sphere crystals and finds that the rate at which they form depends also on the concentration and temperature of the mixtures. These crystalline bodies are not formed in Pickering's bordeaux even after continued standing. This may be due either to a lack of sufficient concentration in the precipitate or to the absence of lime particles around which the copper may crystallize readily. This property of the lime-water-bordeaux is a valuable one in field use, and Pickering has not emphasized it as strongly as he should.

IV. BORDEAUX POWDER AND PASTE

Manufacturers of bordeaux mixture sell it in the form either of powder or paste, usually combined with some insecticide such as arsenate of lead or paris green. Since many persons with but little spraying to do buy these mixtures, it may be well to point out their advantages and limitations.

Bordeaux powders are practically useless as a fungicide, because during the drying process the copper films are changed physically. Even when mixed again with water they do not regain their shape and covering properties. Their insecticide content is not injured in the drying process, which perhaps is fortunate since bordeaux powder is valuable only as an insecticide. Indeed, the finely divided bordeaux precipitate serves only to dilute the insecticide which, of course, costs an abnormally high price.

The reason why the value of bordeaux is impaired by drying is apparent. The colloidal films present in the freshly made mixture collapse and shrivel to such an extent that they possess none of the ability they had when fresh to apply themselves closely to and to dry to the surface of the leaves of sprayed plants. The process of drying completely changes the condition of these colloidal membranes; and the powder resulting from their desiccation is physically an entirely different substance.

Bordeaux paste costs very much more than does the same amount of bordeaux made at home; but if one has only a small plot of potatoes or but a few trees, its purchase may be advisable.

The commercial bordeaux paste mixtures on the market do not contain the copper spheres, at least none were found in the several samples examined. The process of their manufacture is not made public, neither does Pickering tell the method used by the firm preparing his mixture in a paste form. However, it is probably similar to that employed in the preparation of Pickering's bordeaux mixture, lime-water being used and the precipitate separated out by filtration. At any rate the precipitation membranes in their original condition are present in the paste as it is found on the market, and the mixture, after the addition of water, is physically the same as freshly prepared bordeaux and should be equally serviceable if not over-diluted.

V. PRESERVATION

The physical condition of a freshly made bordeaux mixture can be conserved by the use of sugar. Attention was called to this method by Kelhofer (23), although a German chemist named Rumm previously had taken out a patent on a mixture in the making of which the same chemical principles were involved. Kelhofer's recommendation was to add 100 grams of sugar to each hektoliter of the bordeaux mixture which it was desired to preserve. This is equivalent to 3.57 ounces to 10.57 quarts, or about four pounds to a barrel of fifty gallons. According to Kelhofer, the sugar and lime unite to form a lime saccharate and this latter compound further combines with the copper in a double chemical union. Kulisch (26) has found that by using this method bordeaux made in May could be kept all summer in as perfect condition as the fresh mixture, as shown by its efficiency in preventing the mildew of the grape. The small loss in adhesiveness brought about by the addition of the sugar is not of sufficient importance to be a serious practical deterrent to the use of this method of conservation.

Several trials made at this Station indicate that the amount of cane sugar recommended for use is unnecessarily large, at least for the 5-5-50 mixture so frequently used in this country. Not only is sugar expensive, but, also, it seems to act as a reducing agent if the mixture is kept any length of time. Glucose also serves as a preservative, but in a yet more pronounced way reduces copper.

Twelve quart jars were filled with freshly made 5-5-50 bordeaux mixture, to which were added either glucose or saccharose (cane sugar) in ½, 1, 2, 3, or 5 gram lots, two of the jars being left unsweetened as checks. Noticeable reduction was evident after 48 hours in all the jars except the 1½ grams saccharose and the ½ gram glucose jars. The blue

precipitate appeared to retain its physical condition and an examination under the microscope showed none of the sphere crystals found in the old mixtures. The supernatant liquid in all of the jars to which the saccharose had been added was blue in color, due undoubtedly to the double lime saccharate in solution. This hue was most pronounced in the jars containing the five grams addition and hardly was noticeable in that containing one gram. Saccharose must exert some effect on the physical condition of the films, but it is difficult to determine its nature. Possibly the strength of the membranes is decreased, enabling them to expand more easily, thus inhibiting contraction and crystallization of the enclosed substances and of the membrane itself. Butler (7) claims that cupric oxide is formed after a long time, but the writer has observed no physical changes following the use of sugars.

The addition of a gram of sugar to a quart is equivalent to the use of about 200 grams to a barrel, or a little less than a half pound of sugar. The appearance of glass quart jars of bordeaux, three months after treatment, is shown in plate IV, fig. 1. The plate speaks for itself. Clearly it is now possible to make the spray material for an entire season once for all and to keep it in storage, or to preserve a barrel of the mixture if weather conditions become unfavorable for its use after it has been prepared. This is an important consideration in these days when copper sulphate costs fabulous prices. Furthermore, it is possible that even the present high price of sugar need not deter one and that the blacker grades of maple sugar might be used; but whether or not its impurities would affect injuriously the quality of the mixture, the writer cannot say.

VI. FUNGICIDAL EFFECTS

The earlier applications of this mixture were made entirely with a view of preventing fungus disease on the sprayed plants. The chance observation of Millardet in 1882 upon the grape foliage and fruit which had been sprinkled with a mixture of lime and copper sulphate in water in order to deter marauders from eating the ripe grapes, had far reaching consequences in the prevention of fungus attacks on cultivated plants. Millardet rightly attributed this prevention of disease largely to the action of the copper in solution in the mixture and the experiments under his direction (Millardet and Guyon, 31) were designed to determine the rapidity with which this copper became available under ordinary atmospheric conditions. As already described, freshly made mixture contains an excess of calcium hydroxid which must be neutral-

ized before the copper will pass into solution. It is obvious that this must be brought about either by the carbon dioxid of the air or by other gases which are acid when combined with water. This neutralization of the excess of lime is hastened by rain, dew, fog and damp air and is retarded by dry warm air. As their initial experiment was of great importance and has served as the basis for statements in many of the textbooks and bulletins dealing with the use of bordeaux mixture, and as it is not readily accessible, the original matter (translated) is quoted in full:—

"On April 10 the following types of the mixture were prepared:

	No. 1	No. 2	No. 3	No. 4	No. 5
Copper sulphate, grams	16	15	15	15	15
Pure lime, grams	3.36	3.36	6.72	13.44	26.88
Distilled water, liter	1	1	1	1	1

"As may be seen, in No. 1 there is one gram of copper sulphate in excess. No. 2 contains exactly the quantity of lime necessary to precipitate the copper sulphate. Tests made immediately after its preparation showed that there was no excess either of lime or copper sulphate. No. 3 contained twice as much lime as No. 2, while No. 4 contains twice as much as No. 3. Finally No. 5, which is the formula in common use, contains twice the quantity in No. 4. These five mixtures were dried at once in a desiccating oven at 36 degrees C.; then the products were pulverized in a mortar. Ten grams of each of the mixtures were spread in a thin layer between two Berzelius papers, supported below by a fine silk gauze, and were laid thus over five glass dializing jars having equal sized mouths. The necessary precautions were taken to prove whether the paper and the gauze were capable of absorbing any considerable amounts of copper.

"On the evening of April 12 the five jars were exposed in a garden in an open place. After April 13 they were watered simultaneously by all the rains that fell. When it did not rain for some little time, each jar was given an equal quantity of rain water, which had been collected in advance. Each dializer was placed separately in a funnel draining into a test tube in which any water poured on the apparatus finally collected. Every two or three days the water from each of the five apparatus was removed simultaneously and analyzed separately. The following shows the order of appearance of copper in the water which had drained through the mixture:

Mixture No. 1 ....April 17
Mixture No. 2 ....April 19
Mixture No. 3 ....April 24
Mixture No. 4 ....April 25
Mixture No. 5 ....April 30

"Other tests of the same nature, made by washing with rain water leaves of spindle wood and boxwood (Buxus) previously sprayed with these same five types of bordeaux mixture, gave analogous results. In general, the copper appeared in the water which had washed the leaves more quickly when the mixture contained less lime."

The conclusion drawn from this trial was to the effect that the copper in the mixture was not available until it had been exposed for a period varying from five to eighteen days.

CONCERNING THE SOLUBILITIES OF THE COPPER SALTS

These statements, although perhaps not the data on which they were based, seem to have remained unchallenged for a generation, notwithstanding the fact that they are opposed to the observations of everyone who has used the mixture. They mean simply that the mixture has no fungicidal properties until it has been largely washed off the foliage, while it is a well known fact in practice that bordeaux protects as soon as it is applied and that one of the best times to apply it is just before a rain, so that it may protect the leaves from the penetration of the fungus germ tubes.

Both Crandall (9) and Pickering (34) have shown that there was some error in Millardet's reasoning and that free copper is present almost immediately after spraying. Crandall repeated his experiments, spraying young apple trees in pots and collecting the drip from them in large stone jars. Tree 49, sprayed on August 23, may be taken as typical. A shower lasting twenty minutes came within twelve hours after the spraying, and the collected water contained 2.8 milligrams of soluble copper per liter. Determinations are as follows:

		Soluble constituents of drip waters			
		Copper		Alkalinity in terms of calcium oxid	
Date	Amount of drip; liters	Total milligrams	Milligrams per liter	Total milligrams	Milligrams per liter
August 24	1.64	4.6	2.8	45.1	27.5
August 27	13.92	74.4	5.3	154.8	11.1
September 3	1.25	104.4	83.5	Too dark	...
September 12	1.70	61.3	36.0	Too dark	...
September 20	1.80	9.8	5.4	Too dark	...
September 22	9.14	184.4	20.1	Too dark	...
September 26	0.80	41.0	51.2	Too dark	...
September 27	5.45	53.4	9.8	Too dark	...
September 29	10.60	238.8	22.5	Neutral	...
October 1	15.88	93.1	5.8	Neutral	...
October 6	3.02	72.4	24.0	Too dark	...

In the majority of cases the drip water was not collected until from five to seven days after the application had elapsed, but the conclusion reached by this investigator was that the copper was present constantly in solution in small but measurable quantities, varying from one part in 11,000 parts to one part in 170,000 parts. It is to be regretted that more drip water was not collected on the day following the spraying. Crandall further noted the slowness of the solubility of the copper deposited in the bordeaux. The amount of copper taken from a tree 75 days after spraying was more than that taken only seven days after, and the leaves still were coated. It must be noted here that the trees were given three heavy sprays with intervals between them only long enough to permit the applications to dry.

Pickering (34) agrees with Crandall, but seems to have made no experiments on the immediate solubility of the copper. He explains Millardet's and Guyon's failure to get free copper before five days as follows:— "In Millardet's and Guyon's experiments, the water supplied would form lime water so long as any free lime was there, and this would decompose any copper sulphate which might be liberated. But when bordeaux mixture is sprayed onto foliage it dries up, and the particles of basic sulphate and of lime will become separated from each other; hence, when the former are attacked by the carbon dioxid there may be no lime particles close enough to decompose the copper sulphate formed." He illustrates this point by wetting a piece of filter paper with a solution of potassium ferrocyanide, allowing it to dry, and then letting a drop of bordeaux fall on it. As long as the paper is wet from the bordeaux mixture no red color shows as the lime water can reach the copper compounds, but as soon as the drop is dry the red color appears in patches. Around the lime particles the paper is colorless. The same phenomenon can be demonstrated even better by drying a drop of bordeaux which is spread out thin on a glass slide, and then converting the copper by means of a potassium ferrocyanide solution. Practically the whole bordeaux film becomes red at once (fig. 8) even if the slide is not exposed to the air further than to dry the drop. The particles of lime may be seen imbedded in the film. The explanation goes back to the physical properties of the mixture. Each of the saccules formed when the copper sulphate solution and milk of lime come together is likely to contain some particles of lime, but the bulk of its contents is lime water. This evaporates and the small amount of residual calcium hydroxid is neutralized by the carbon dioxid of the air. These films in drying are very likely to overlap, but the

lime granules still lie some distance from each other. If this surface is now moistened, the water in contact with the film contains soluble copper, while that around the lime is strongly alkaline. In freshly applied bordeaux the copper in the thinner portions of the films and in those furthermost removed from the lime particles possesses the strongest fungicidal value and enters into solution first, whereas in proportion as the applied mixture ages, more and more of the copper will be removed by rain and dew until only fragments of the original layer remain and, theoretically, these would be those located around the lime particles. As a matter of fact, however, these particles of lime, unless they are small, present more surface to the beating of the rain than the smaller film fragments and, consequently, are more likely to be torn from the leaf. This gradual solubility of the copper is one of the two outstanding features of the ordinary bordeaux mixture—the other feature is its adhesiveness; and while Pickering's claim for his bordeaux compound that its copper is much more soluble and immediately available than that in ordinary bordeaux is valid, it is also true that it will have lost its copper when the mixture made in the usual way is still on duty, giving off enough copper to prevent the growth or penetration of fungi.

The above discussion of the solubility of the copper salts in the mixture and of the physical and chemical form which the solution takes, has been introduced to show how useless has been much of the experimentation on the fungicidal action of the compound. Any experiments on the dilution of bordeaux mixture with water made with the view of testing its fungicidal or toxic properties in an aqueous solution practically are valueless (Dandeno, 11), as they do not reproduce in any detail the conditions that are found on a foliage leaf when a fungus spore and a layer of bordeaux mixture come together. The only thing such experimentation can show is the effect of a fairly alkaline medium on the spores or seedlings tried. The only method by which natural conditions can be reproduced is to dry dilutions of the mixture on slides and then to attempt to germinate the spores in a drop of water over this layer. If there is any soluble copper present or if the lime is still in excess, this will be shown by the water in this drop. In this way the chemical changes that occur in the films on slides are similar to those occurring in nature on the foliage of plants. If the bordeaux mixture is not dried to the slide but remains suspended in water, the lime contained in the films will continue to go into solution and the drop of the solution will remain alkaline and without free copper.

The method used in the work was as follows. Half a cubic centimeter of the required dilution was pipetted on the slide and spread out into a circular area of one-half to one square inch. After drying thoroughly, about the same amount of water was placed on the dried film and in this the spores were placed for germination. A very weak solution of dextrose was used in a few experiments but water was found to answer the purpose as well. A method somewhat similar to this has been used by Wallace, Blodgett and Hesler (44) in their study of the fungicidal value of lime-sulphur preparation, in which some of the dilution to be tested was sprayed over a slide, allowed to dry and the spores then sown in water over this layer. Barker and Gimingham (5) attempted to secure analogous results by passing carbon dioxid into the mixture. Such a procedure results only in neutralizing the alkalinity of the entire mass of bordeaux and is open to the same criticism which may be lodged against the attempt to determine the toxicity of bordeaux by the simple dilution of the mixture itself.

In the following experiments *Ustilago levis, U. nuda, Sclerotina cinerea* and *Phytophthora omnivorum* were used at various times as the fungi with which to test the fungicidal properties of the mixtures. The *Ustilagos* and the *Phytophthora* are supposed to be fairly sensitive to copper solutions, judging by Wüthrich's (46) experiments on the same or similar species, in which he found that one part of copper sulphate in 8,000 entirely prevented germination. On the other hand, the great resistance of *S. cinerea* to copper salts is mentioned by Frank and Krüger (17), and other authors claim that a one percent solution of copper sulphate is necessary in order to prevent its growth.

EFFECT ON SPORE GERMINATION

1. *Effect of exposure of dried bordeaux mixture on its fungicidal action.* The following experiments will illustrate this effect:—

(a) 0.5 c.c. of a 1 to 100 dilution of 5-5-50 bordeaux was dried to a slide and left unexposed.

(b) Same as (a) but slide exposed over a damp night.

(c) Same as (a) but Pickering's bordeaux used instead of the 5-5-50; unexposed.

(d) Same as (c) but exposed.

The spores of *Phytophthora omnivorum* were sown in a little water on the slides and kept under a belljar over night. Tests (a) and (b) both showed fair germination of spores in the liquid with no differences visible as between the two, whereas in tests (c) and (d) prac-

tically no germination whatever occurred. The germination of the checks was excellent.

2. (a) 0.5 c. c. of a 1 to 100 dilution of 5-5-50 bordeaux was dried to a slide and left unexposed. *Phytophthora omnivorum* conidia were placed on it in water.

(b) Same as (a) but slide was exposed for three days, one of which was rainy, before it was brought in and the *P. omnivorum* conidia placed in the water on it.

(c) Same as (a) except that *S. cinerea* was used as the test fungus.

(d) Same as (b) but exposed for three days as in (c).

The conidia lying against the bordeaux in tests (a) and (c) were killed, and many of the floating conidia also were destroyed. No marked differences were observed as between (b) and (d), and no conidia showed germination. The germination of the checks was excellent.

3. (a) 0.5 c. c. each of 0.1 and 0.01 dilution of 5-5-50 bordeaux were dried to slides and exposed for two days. Rain occurred during all of one night, but otherwise the weather was clear.

(b) Same as (a) but unexposed. Spores of *Ustilago levis* and *Sclerotina cinerea* were sown.

Slide	*Ustilago levis*	*Sclerotina cinerea*
(a) 0.1, 5-5-50	No germination.	No germination.
(b) 0.1, 5-5-50	A few germinations of floating spores.	A few germinations of floating spores.
(a) 0.01, 5-5-50	No germination.	Good germination.
(b) 0.01, 5-5-50	A few germinations of floating spores.	Good germination.

Clearly there is very little difference in this respect between exposed and unexposed bordeaux. As shown in experiment 3 there may be a little free copper which was active but this would come almost within the range of variation of toleration of the spores to the poison.

4. *Effect of the water from bordeaux films on spore germination.* As has been shown by Crandall (9) free copper always is present in the water which comes from the sprayed plants. It is very questionable if this has the slightest effect on fungus growth, as can be seen by the following experiments:—

Slides containing spores of *P. omnivorum* and *S. cinerea* were sown on:—

(a) Supernatant water from exposed bordeaux.

(b) Supernatant water, diluted ten times, from exposed bordeaux.

(c) Control.

The germination was good in all of these trials. No difference could be observed either in number of germinations or in length of germ tubes.

Clearly the water derived from the bordeaux films has little germicidal power. Nearly all the soluble copper must lie close to the films from which it is derived, since when located at any distance it is neutralized by the calcium hydroxid originating in the lime particles. That the amount of fungicide available in a drop of water is not very large, with the exception of that in contact with the spray mixture, is further illustrated by the following experiment on lime-sulphur mixture:

5. *Effect of water from lime-sulphur spray on spore germination.* Spores of *Sclerotina cinerea* were sown on the following slides:—

(a) Check.

(b) 0.5 c. c. of 1 to 50, 1 to 100, or 1 to 200 dilutions of lime-sulphur dried to slide.

(c) 0.5 c. c. of a 1 to 200 or a 1 to 400 dilution of 5-5-50 bordeaux, dried to slide.

No germination occurred in the lime-sulphur lot among the spores lying among the spray mixture, but there was some germination in the floating spores although the germ tubes were only one-half the length of those of the check.

No germination occurred among the spores resting on the bordeaux, but a few floating specimens put out short germ tubes. The spores were washed off into a weak solution of dextrose but practically no germination ensued, showing that the spores had been killed by contact with the fungicides.

Germination in the check was good, germ tubes being 10 to 15 times longer than the diameter of the spore.

6. *Germination of spores in contact with dried lime-sulphur mixture.* Spores of *Sclerotina cinerea* were sown on slides on which 0.5 c. c. of a 1 to 1,000 and 1 to 10,000 dilution of common lime-sulphur had been dried in spots of about a surface area of a square inch. The spores on the 1 to 1,000 dilution did not germinate at all. Those on the 1 to 10,000 dilution produced short germ tubes, only about one-third as long as normal (figure 5).

7. *The effect of lime water and alkaline solution on spore germination.* Two lots of 5-5-50 bordeaux mixture were titrated with N/10 hydrochloric acid. It was found that about 0.36 c. c. of normal acid was required to neutralize the alkalinity of 1 cubic centimeter. Then milk of lime was made up so as to titrate about the same as the un-

Fig. 5. Germ tubes of *S. cinerea* in nutrient solution and in nutrient solution in contact with dilute lime-sulphur mixture dried to the slide.

combined lime in 5-5-50 bordeaux mixture (0.35 c. c.N/1 acid per c. c.). Dilutions of 1 to 10, 1 to 100 and 1 to 1,000 then were made and the spores of *Sclerotina cinerea and Phytophthora omnivorum* were placed in the drops, together with the addition of a very little dextrose solution, without allowing the latter to dry down on the slide. These slides were placed in a rack under a belljar together with a dish of concentrated potassium hydroxid solution to absorb the carbon dioxid. The following morning the spores in the check and in the 1 to 1,000 dilutions showed many germ tubes while the germination in the 1 to 100 dilutions was slight in the case of the *Phytophthora omnivorum* and in that of the *Sclerotina cinerea* the tubes were very short, one to two times in length the diameter of the spores. In the 1 to 10 dilution there was no apparent germination, but the spores apparently were normal and had not been killed. In another trial the lime was used in 1 to 1, 1 to 2 and 1 to 10 dilutions. No germination occurred in any of them even after 24 hours. The lime water, and as much of the lime as possible, were drained off and the spores placed in a weak dextrose solution to which a few drops of lactic acid had been added. No germinations resulted. The contents of the spores were found to have become plasmolyzed, indicating that their long bath in the alkaline solution had killed them.

8. *Effect of distance of spots of the mixture on the germination.* Some slides were made up with lines of Pickering's bordeaux, just at

neutrality, while with others the mixture was put on in the form of checks (fig. 6). About two-thirds of the surface was covered in the checked slides and about one-third in the slides with lines. Pickering's

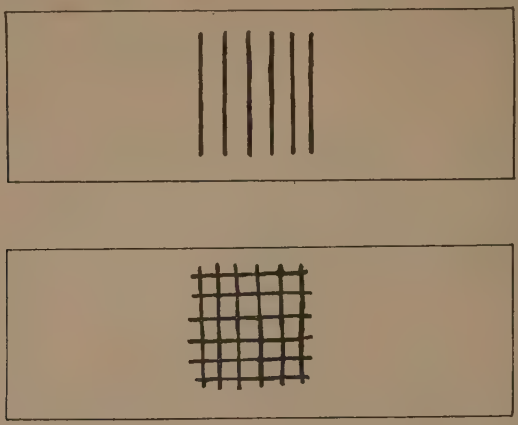


FIG. 6. Arrangement of bordeaux on slides.

soluble bordeaux was used so that the copper might be available immediately. The lines of bordeaux mixture were covered with plain agar and conidia of *Phytophthora omnivorum* sown on it. The slides then were placed over night in a saturated atmosphere. Not as large a percentage germinated on the finely checked as on the coarsely lined slides and the mycelium seemed to be less profuse. The same experiment was tried using *Sclerotina cinerea* as the test fungus and similar results were attained. The germ tubes were a little shorter in the closely checked slides. The spores of *Sclerotina cinerea* also were sown directly on the lines and checks of bordeaux in a very weak dextrose solution, but no germination resulted. The lines were about two millimeters apart. The following experiment will serve to show the cause of the lack of germination.

Bordeaux mixture (5-5-50 without dilution) and Pickering's bordeaux (concentrated precipitate at the bottom of the vessel) were placed on the slide in the form of dots, in some instances one being placed on a slide and in others two. The drop of liquid containing the spores was placed either between the two spots or at one side of the single spot. The results secured with the Pickering's bordeaux were most striking, as the following diagrammatic drawing will show:—

Fig. 7. Germination of *S. cinerea* near a drop of Pickering's bordeaux (diagrammatic). The regularly spaced marks on the horizontal line indicate tenths of millimetre from the drop.

The spores in close proximity to the spot apparently were dead and no germinations were visible. Part of those a little farther away put out germ tubes, but these were much shorter than those still farther removed from the influence of the copper. The distances indicated on the diagram are 0.1 of a millimeter. The results might be summarized as follows:—

0.8 to 1 m. m. from bordeaux spot. No germination.

1 to 1.5 m. m. from bordeaux spot. About 25 percent germination; tubes three to five times the diameter of the spore.

1.5 m. m. from bordeaux spot. About 50 to 75 percent of the spores germinated, the germ tubes being 20 to 30 times the diameter of the spore.

In similar experiments with 5-5-50 bordeaux the results were less clear cut than with Pickering's mixture. It was applied to the slide in the form of drops and lines. The great excess of lime complicated the action of the copper compounds. If the drop of water between the bordeaux spots covered them completely and dissolved some of the lime, the germination of the spores was prevented entirely, the result being due probably to alkalinity and concentration. If the drop of water touched the drops of bordeaux only slightly, the range of germination was about the same as in the spots of Pickering's mixture. Spore germination between lines and checks of the mixture was prevented, even when these were four to five millimeters apart. It was observed, however, that there were some germinations at one end of the spot of water at a distance of eight to nine millimeters from the nearest line of the mixture.

From these results the conclusion may be drawn that bordeaux protects the sprayed foliage not only over the immediate spot upon which it rests but over any unsprayed portions not more than a millimeter away.

The observations of Aderhold (1) to the effect that the spores of *Fusicladium dendriticum* germinate between freshly applied spots of bordeaux mixture on apple leaves is interesting, but he gives no information as to the distance between these spots. He believes, however, that the germination of the spores on sprayed leaves is no test of the efficiency of the bordeaux, but that its fungicidal effect is shown during the formation of the infection tube or during infection itself.

Observations were made also as to the position of the germ tubes with reference to the spots and as to the direction which later they assumed, in the hope of discovering either positive or negative chemotropic effect of the soluble copper. Neither the appearance nor the direction of the germ tubes was altered by the presence of the bordeaux spots.

9. *Effect of the presence of copper wire on germination of spores.* A series of cultures on glass slides was made of *Phytophthora omnivorum* and *Sclerotina cinerea,* a quarter of a cubic centimeter of water being placed on each slide. Pieces of polished copper wire, gauze No. 32, one to five centimeters in length were placed in the various cultures. The five centimeter pieces of wire were rolled up into the form of a loose spiral about a centimeter across, and the two centimeter pieces were bent in the form of the letter U, while the one centimeter piece was put in the cultures straight.

The results, after 18 hours, were as follows:—

Five centimeter wire. *Phytophthora omnivorum* showed much abnormal germination while but very few spores developed normally.

Sclerotina cinerea spores had not germinated among the coils of the wire nor at a distance of about twice the width of the wire away from it. The germinations near to the wire were with short tubes, while at some distance were as long as those in the control.

Two centimeter wire. *Phytophthora omnivorum* germinated in about the same manner as above; but a great number of the zoospores seemed to lie along the wire. Whether this was a chemotropic effect or due solely to physical forces was not determined.

Sclerotina cinerea showed no germination within the U-shaped loop of the wire, but outside the germ tubes were at first shorter and, still further away, normal.

One centimeter wire. *Phytophthora omnivorum* spores were unaffected as to their germination.

S. cinerea germinated in much the same manner as cited above.

Apparently ordinary copper wire will inhibit germination in its immediate neighborhood and will prevent the formation of full length germ tubes at a slightly greater distance.

10. *Effect of copper wire and of bordeaux on Spirogyra.* Spirogyra is one of the algae most sensitive to the presence even of minute traces of free copper of any of the copper salts. It was thought, therefore, that the effect of bordeaux dried to slides on fungus spore germination could be checked with its action on a much more sensitive alga. A series of crystallizing dishes containing each about 100 cubic centimeters of water were used and in them were placed the following objects together with a little Spirogyra:—

1. Check.
2. 1 bordeaux slide; 1 to 200 cc. of 5-5-50.
3. 2 bordeaux slides; 1 to 200 cc. of 5-5-50.
4. 3 bordeaux slides; 1 to 200 cc. of 5-5-50.
5. No. 32 copper wire; 4 cm. long.
6. No. 32 copper wire; 8 cm. long.
7. No. 32 copper wire; 16 cm. long.
8. No. 32 copper wire; 32 cm. long.

These trials were started in the afternoon. The following morning, the alga in No. 8 was not quite as bright green as at the outset, and in three days it was brown and dead. A color change was manifested in No. 7 in three days. Numbers 5 and 6 showed a browning especially on the side next to the wire and the liquid was brownish, but the plants were not all killed. Numbers 2, 3, and 4 were not materially affected although the water was somewhat discolored and the plants on the underside were not as bright green as was the check. However, this appearance did not become at all noticeable until five days had elapsed.

The check remained normal throughout. The experiment was repeated with the following arrangement of the dishes, bordeaux slides and copper wire.

1. Check.
2. 2 slides; 1 to 200 cc. of 5-5-50.
3. 1 slide; 1 to 10 cc. of 5-5-50.
4. 2 slides; 5 cc. 5-5-50.
5. 1 to 200 cc. 5-5-50 spread over bottom of dish.
6. No. 32 copper wire; 4 cm. long.
7. No. 32 copper wire; 8 cm. long.
8. No. 32 copper wire; 16 cm. long.
9. No. 32 copper wire; 32 cm. long.

No. 8 was dead within 48 hours and No. 5 succumbed almost as promptly. Number 9 and 7 seemed to show much the same effect

but to a less degree, losing color on one side only and this after some days. The algae in No. 9 may have been originally in a somewhat healthier condition than their mates. The groups in Nos. 2, 3, and 4 showed the effects of the copper on the side lying against the bordeaux slides.

A similar experiment was tried on *Zygnema,* but the results were less satisfactory. This alga seemed to be much less sensitive to traces of free copper than *Spirogyra.* The crystallizing dishes were arranged in the following manner:—

1. Check.
2. 1 cc. of 1 to 100 cc. Pickering's bordeaux.
3. 1 cc. of 1 to 200 cc. Pickering's bordeaux.
4. 1 cc. of 1 to 1,000 cc. Pickering's bordeaux.
5. 1 cc. of 1 to 100 cc. 5-5-50 bordeaux.
6. 1 cc. of 1 to 200 cc. 5-5-50 bordeaux.
7. 1 cc. of 1 to 1,000 cc. 5-5-50 bordeaux.
8. No. 32 copper wire; 5 cm. long.
9. No. 32 copper wire; 10 cm. long.
10. No. 32 copper wire; 20 cm. long.

The bordeaux was spread over the bottom of the dishes and then allowed to dry, following which 25 cc. of water were poured into each dish and a small tuft of *Zygnema* added. The dishes were placed in diffused light. After 24 hours the check was still a bright green, except the side of the tuft turned towards the copper wire or the dried bordeaux. The 1 to 200 and 1 to 100 dilutions of Pickering's bordeaux affected the alga in about the same manner as the longer pieces of copper wire while the 1 to 100 and 1 to 200 dilutions of the 5-5-50 mixture turned it a much darker brown. The strongly alkaline properties of the latter mixture must be remembered.

A similar trial was made in which slides to which 0.5 cc. of 1 to 400 and 1 to 200 dilutions of 5-5-50 had been dried were compared with pieces of copper wire, 15 to 25 cubic centimeters in length. Part of these slides had been exposed for 48 hours in clear weather. The longer (25cm.) coils of wire affected the *Zygnema* more than either the 1 to 400 or 1 to 200 dilutions of the bordeaux, whether exposed or not.

GENERAL DISCUSSION

In the light of the result of the experimental trials thus far outlined, as well as those of Crandall (9) and Pickering (34), it is of interest to recall the suggestions made by Swingle (42) more than 20 years ago as to the method by which bordeaux mixture prevents fungal infection of plants. They were as follows:—

"(a). The spores may be prevented from germinating by inhibitory action. (b) The protoplasmic content of the spores may be killed outright in a short time before germination has commenced. (c) Through negative chemotropic action of the copper hydroxid the germ tube may be prevented from entering the plant. (d) The germ tube may be so weakened by copper in solution as to be unable to enter the host plant. (e) The germination tubes may be prevented from growing or be killed only on contact with solid particles of copper or its compounds, or with the cuticle or other parts of the host impregnated with copper. (f) The germ tube may be so much injured soon after germination as to cease growing before attempting to effect an entrance into the host plant, or may be killed outright soon after appearing. (g) The effects of the copper contained in bordeaux mixture may be exerted at a later stage of development of the fungi. (h) The presence of a thick coating of copper salts might impede the fruiting of a fungus already within the tissues of the host plant."

It may be worth while at this time and in the light of our knowledge of today to examine some of these possibilities in the order in which Swingle presents them.

(a) In a number of the experiments discussed in this bulletin the spores were prevented from germination; but whether this result was due to an inhibition of germination or to the death of the spores still is an open question. However, there is this much to be said, that in every case where the spores were washed off and placed in nutrient media they failed to germinate. A large proportion of the spores which might fall in the moisture on a leaf which is well sprayed with bordeaux would lie against the thinner membranes containing little lime and giving off small doses, increasing in concentration as the film itself is reached (figure 8), and as the spores lie in direct contact with this film they get the full benefit of any such solution. The border line between the inhibition of germination and the death of the spores probably is a narrow one and is dependent almost entirely upon the length of the time during which the spore remains in contact with the layer of fungicide. An exposure of only a few minutes probably is all that is necessary with the more sensitive fungi, varying with the fungicide used and with the completeness of covering it afforded the plant. In the experiments with the dots of bordeaux mixture (p. 24) the writer fails to find evidence that the soluble copper salts released exerted any chemotropic influence either positive or negative on the place of formation or the later direction of the germ tubes (fig. 6).

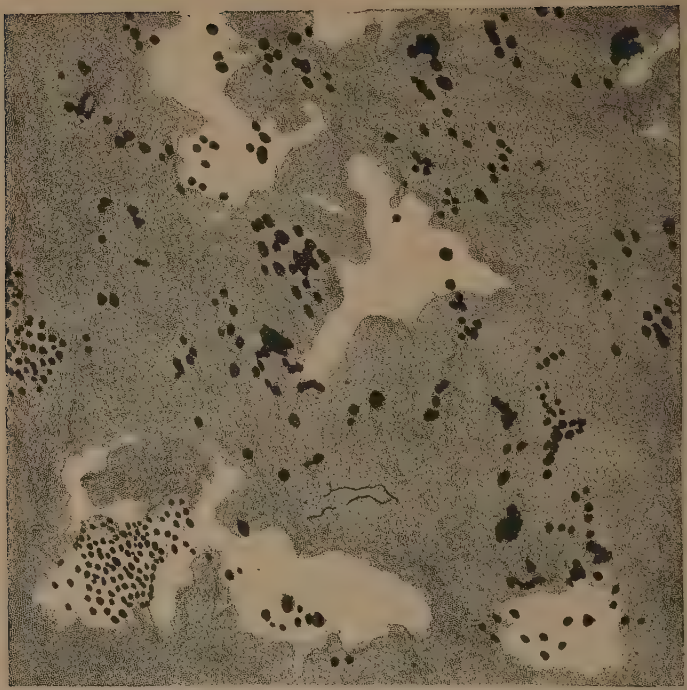


FIG. 8. Portion of slide covered with bordeaux. Lighter shaded portions show the copper reaction; dark bodies are particles of lime.

Swingle's suggestion, that the germ tube may be so weakened by the copper in solution that it may be prevented from entering the host plant, seems plausible in the light of the experiments now under review. Even in instances where the fungicide was not strong enough to kill the spores or to inhibit their germination, this was delayed and germ tubes were shortened. Either of these occurrences in most instances would be fatal; the plant thus produced would die, usually a result due to the evaporation of the drop of water in which it lay before it could germinate or could develop a tube of sufficient length to penetrate the epidermis of the host plant. It would seem from this that the absorption of very minute quantities of copper salts by the fungus serves to retard its growth process. In view of the fact that spore germination is brought about largely by the absorption of water and the swelling of the material already stored in the spore, the water containing traces of copper salts is partly inhibited by the plasma mem-

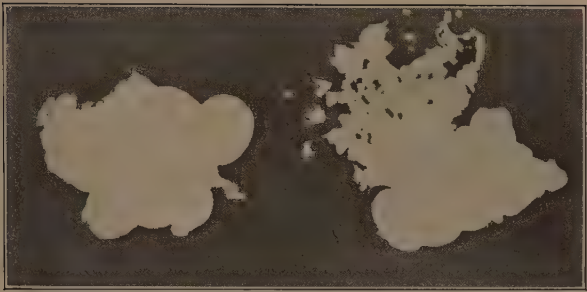
brane from entering the cell. This action must be still weaker than that assumed by Nägeli (32) as due to oligodynamic forces. Nägeli assumed that there were two types of poisoning, chemical and oligodynamic. The chemical type is brought about by the effects wrought by an appreciable amount of the toxic substance in solution. In copper sulphate this would mean for *Spirogyra* or for the ordinary fungi, one part in from 1,000 to 10,000 of water. If this solution is diluted still further so that there is only one part of copper sulphate in a million or more of water, the oligodynamic effect is manifested and results in no immediate death of cells but in a disorganization of the spiral bands of chlorophyl of *Spirogyra*. In the fungus cells no such visible internal disorganization can show how the poison is acting, but the retarded germination and short germ tubes make it apparent that the life processes in some way have been interfered with and are not progressing normally. This observation is confirmed by the growth on check slides.

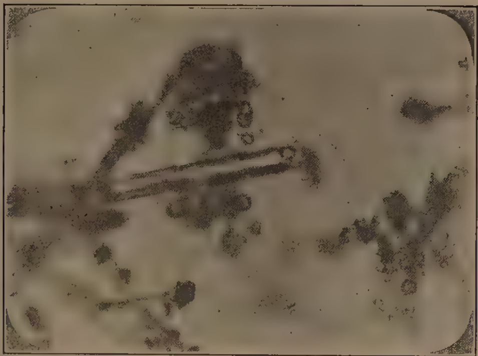
(e) Swingle's fifth suggestion, that the germ tubes may be killed by growing against copper particles or against cell walls impregnated by copper, does not seem to be important. Such occurrences cannot be frequent. The germ tubes, if formed, hardly would grow in any great numbers within range of the sprayed spots, although a few might thus commit suicide. The presence of copper in the cuticle of sprayed plants has been demonstrated by Dévaux (12), who was able to show that copper was absorbed in sufficient amounts to be detected by means of the spectroscope. This means that the fungus in order to penetrate this cuticle must dissolve this copper-impregnated layer. If other enzyms act as does diastase, according to Ewert (15), the solution of this wall is made still more difficult and it may be almost impossible. The importance of this copper-impregnated cuticle in resisting the attacks of fungi after the bordeaux has been largely washed from the leaf, is apparent. That this protection does not continue throughout the season is unfortunate, for if it did one annual spraying would suffice. In this connection mention must be made of the suggestion of Rumm (35) that the leaves absorb the copper salts in the cuticle and that there it serves as a stimulus, due solely to its presence which influences the life activities of the plant. This author made a spectroscopic examination of the ashes of sprayed leaves from which all the external adhering copper had been washed and was unable to demonstrate the presence of copper. The assumption therefore was that the copper acted only chemotactically on the cells. The work of

Ewert (15) in this connection should be recalled, as he was able to show that copper salts were absorbed and by their action on the diastase affected the removal at night of the starch formed during the day. It is hardly possible that the plant cells could fail to absorb some of the copper, but on the other hand undoubtedly it would be in such dilution that it would have little effect on the fungi. The suggestion of Rumm above referred to, that the plant is made so healthy and vigorous by means of the copper salts that it can withstand fungus attacks, seems in view of the previous experiments to be much less plausible than the hypothesis which ascribes the benefit arising from spraying to the mechanical and chemical protection afforded to the plant externally by the presence of the layer of fungicide.

(f), (g), (h). No evidence has been presented in these trials bearing on Swingle's conclusions thus indicated. It is probable, if we are to judge by the results secured in experiment 8, page 24 and as has already been pointed out, that the presence of copper salts is more likely to retard germination and to inhibit the growth of the germ tube than it is to kill the tube after appearing, unless it should be so unfortunate as to grow towards a particle of the spray solution. The effect of the copper on the growth of the fungus after it has made its entrance into the host plant also must be very slight. If it is only present internally in sufficient quantities in some manner to stimulate the sprayed plant, it is hardly probable that it would be found to be of sufficient concentration there seriously to hinder the progress of the fungus throughout the leaf or stem. The external thick coat of spray mixture, however, would retard seriously the formation of new crops of spores on diseased leaves, especially in those diseases where the fruiting bodies are borne on the upper side of the leaf. For internal diseases, such as late blight of the potato and many of the mildews in which the conidiophores come to the exterior through the stomata, the presence of the fungicide largely on the under surface of the leaf would have small effect in preventing the spread of the disease in this manner.

These highly suggestive theories of Swingle, put forth at a time when little experimental evidence existed as to the method whereby the fungicidal effects of bordeaux mixture were exerted, have been shown by the experiments now under discussion and by those of Barker and Gimingham (4) in the main to be correct. However, the conclusions regarding the fungicidal methods of this mixture at which the





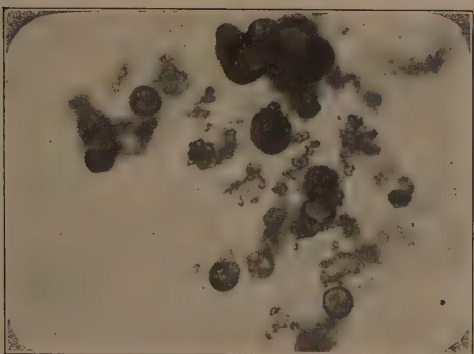


PLATE I. Figure 1. A drop of milk of lime in a solution of copper sulphate forms a gelatinous membrane around itself wherever the two come into contact. If the surface of this membrane is broken, new membranes are formed to cover the new points of contact. Inside these membranes are the lime-particles floating in a saturated solution of lime water.

Figures 2 and 3. Old bordeaux showing the sphere crystals and, in figure 3, the needle crystals, probably of calcium sulphate.

PLATE II. Sprayed and unsprayed rows of Green Mountain potatoes, August 28, 1911. The difference in appearance is due entirely to the destruction of the unsprayed rows by the tip-burn.

PLATE III. Sprayed and unsprayed Green Mountain potato plants, August, 1911.

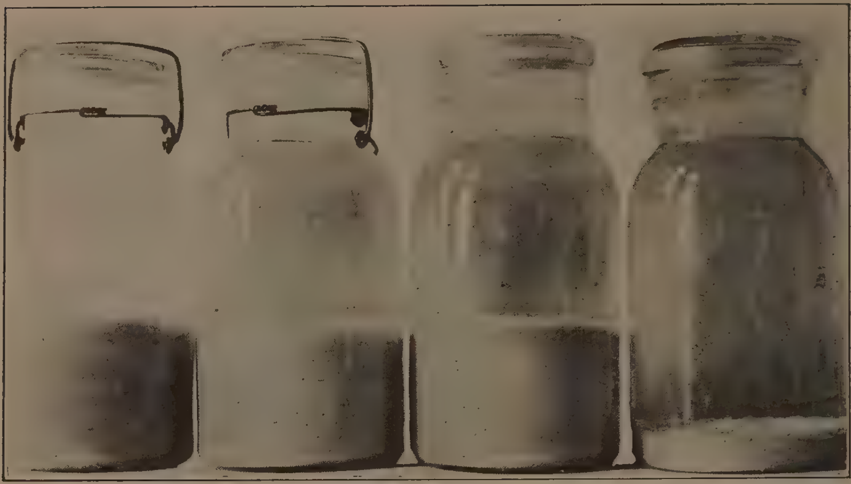


PLATE IV. Figure 1. Jars of bordeaux prepared in April; picture taken in July. No. 1 contained one gram saccharose (cane sugar); No. 2, two grams; No. 3, one gram glucose; No. 4 had had no addition of sugar.

Figure 2. Sprayed and unsprayed rows of Industrie. The German varieties of potatoes are affected even more seriously than the American by tip-burn.

writer has arrived are different in a number of details, some of which thus protected.

After a plant has been sprayed with any of the ordinary bordeaux mixtures, such as the 5-5-50 or 4-4-50 combinations, there is always present on the sprayed foliage a considerable excess of lime in the form of particles, but between these are located colloidal copper membranes giving off free copper as soon as they are moistened. If water falls on such a leaf in the form of drops in sufficient quantity to produce spore germination, the mass of the water is alkaline. This alkalinity is probably sufficient to prevent the germination of any of the ordinary fungus spores. If the water drains off the leaf and only a thin layer remains, that portion on the leaf surface between the lime particles—unless the latter are very close together—holds some soluble copper in it, while that in close proximity to the particles is strongly alkaline. If a fungus spore falls on this moistened leaf it will be killed if it falls in the portion covered by the bordeaux, while its growth will be inhibited and it may die from plasmosis if it should be in the immediate vicinity of some of the lime particles. The leaf may not be entirely covered by the mixture, but if spores fall on it in the vicinity of the sprayed area one of three things may happen. If it is very near to such a spot it may be killed by the copper; if it is a little farther away, its germination may be inhibited; while if it is still farther away, its germination may be retarded and the germ tube may be shorter and probably not as vigorous as it normally would be owing to the absorption of traces of the poison. The distance the sprayed spots may be apart and still prevent the successful germination of the spores has been determined by the preceding experiments as at least a centimeter for some spores. It is not absolutely necessary therefore that every square centimeter of the leaf should be covered, although it is better if possible that it should be are quite important.

The importance of the fungicidal properties of lime and the slow solubility of the copper salts formed in bordeaux mixture cannot be too strongly emphasized, for it is upon these two facts that the long continued efficacy of the ordinary mixture depends. The copper salts becoming slowly soluble over the larger part of the sprayed area serve to protect the larger parts of the foliage, but dotted throughout these dried films are the small particles of lime, the double function of which is to prevent too much of the copper salts in their immediate region from becoming too promptly soluble, and to prevent the germination of the spores and the growth of the fungi over the region in which

they lie. The claim is made for the Pickering bordeaux that since it contains no superfluous lime particles, it is fungicidal as soon as it is applied. It should be pointed out, however, as a corollary of this claim that this mixture must be, if valid, inherently defective in that it necessarily cannot remain fungicidal for nearly so long a time as the ordinary bordeaux, because its copper will have passed into a soluble condition and have been washed away by the rain at a time when the ordinary mixture with its excess of lime has some copper still entering into solution to protect the plant. The question seems to resolve itself as follows: the relative desirability of copper salts in considerable quantities immediately available for use and for a relatively short period of time, and of lesser quantities of these salts available at once but distributed over a longer time. Since the ordinary mixtures furnish enough free copper to exercise a fungicidal effect, there would seem to be no reason for shortening the life of an application of the mixture by spraying with a neutral bordeaux. If lasting fungicidal properties are desired, the ordinary bordeaux is better than the neutral bordeaux without lime particles; and for the immediate protection of the sprayed plant it is quite as good.

Another fact to be noted in this connection is that fungicidal preparations kill by contact. While the water coming from the sprayed leaves may show slight traces of copper, it never contains enough seriously to injure the fungus unless the latter is in intimate contact with the moistened sprayed surface. The amount of copper in solution must diminish rapidly in proportion as its distance away from the spray film increases; indeed at the surface of a drop of water its amount would be almost negligible, so far as its action on the fungi is concerned. It is possible that in some of its life processes the fungus spore may secrete soluble substance, for example carbon dioxid, in sufficient quantities close to the sprayed surface to bring about death in any case. Undoubtedly it is true that the fungus would give off carbon dioxid in its growth, but the instances where this would affect the penetration of the fungus are rare, since the great majority of the spores as they lie in water on the sprayed leaves would be likely to be killed or at least inhibited from germination.

The writer believes that the fungicidal action of ordinary bordeaux mixture may be expressed as follows:—

1 The fungus spores are killed by contact, either:

(a) with the bordeaux film, giving off free copper; or,

(b) with the alkaline solution derived from some of the lime particles.

2 The spores are inhibited from germination if they are in the region either of the bordeaux film or of the lime particles. The germination of the spores is retarded if they lie close to either of these substances and may be held back until the drop of water has dried up or a tube is formed which is too short and weak to penetrate the host cell walls.

VII. THE PHYSIOLOGICAL EFFECT OF BORDEAUX MIXTURE

1. Spraying Trials

On certain plants, such as the potato, the physiological effect of bordeaux mixture seems to be quite as important as is its fungicidal effect. The changes in the potato plant brought about in this manner have been noted particularly at the Vermont station and have been made at least half the basis for the plea it has made year after year, that potato growers spray regularly. The late blight is only serious enough in about half the seasons to make the application advisable on that account. Spraying in those seasons in which it does not occur would be lost labor were it not for the fact that a favorable physiological stimulus is exerted on the plant.

It will be understood clearly, of course, that this bulletin is not a discussion of experiments dealing with advisability of spraying for fungus diseases, such as late and early blight. This part of the potato spraying problem was settled long ago, and careful growers spray regularly. The present work deals entirely with the effect of the mixture on the potato plants themselves. The chance occurrence of fungus disease would have been most embarrassing, for it would have been difficult if not impossible to have arrived at conclusions touching the matter now under consideration which would have been beyond cavil. The writer was very fortunate in being able to carry on this work in years when both late and early blights were entirely absent. A better period could not have been selected, even if the weather could have been foretold five years in advance. Disease was a seriously disturbing factor only during one year (1915); and no extended experiments were at that time under way.

The discussion of these physiological changes in the potato plant is based in large part on the field work of the last four years. It is necessary at this point in the discussion to present the results of this field work. The theory that these good effects are due in large part

to climatic conditions was tested by a summer's spraying in Germany. Other phases of the subject were under observation during the same year and following years at Burlington. Furthermore, experiments were made in the garden and greenhouse to determine under controlled conditions the changes induced in the plants by the copper mixtures. The problem is an exceedingly complicated one and the literature is extensive. It has been thought best, therefore, to discuss previous work in connection with the experiments made at this Station rather than to attempt any complete resumé of the literature at the beginning of this section.

German potato growers rarely spray their potato crops for the reason that late blight has not been a serious trouble with them for a number of years and that they never have been able to secure as favorable physiological results from spraying as seem to have been obtained in this country. German farmers are among the most careful in the world; and it is quite safe to assume that if increased potato harvests followed regular spraying under German conditions they would spray.

In order to determine, if possible, the cause of this divergence between American and European results, the writer made a number of spraying trials in Germany during one season. The year was a fairly typical one, although the spring was rather late and cold. As in the case of the Burlington experiments, the writer was fortunate in hitting upon a year when no complications were introduced by late blight. The only changes which occurred in the plants were those wrought by the spraying.

SPRAYING POTATOES IN BURLINGTON IN 1912

Green Mountain potatoes were planted on sandy and clay loam soils in early June. One of the plots was a very light sand while the other was a heavy clay loam, especially at one end which extended across all the rows. The work was carried on under the direction of the then assistant plant pathologist, G. C. Cunningham. The physiological effect of bordeaux mixture on potatoes grown on a heavy clay soil was to be compared with its effect on the same crop grown on a light sand soil. The spring was very wet and late and the plants started slowly. On July 5, when the first spraying was attempted, they were only a few inches high. The same spray was used as in previous years, namely, a 5-5-50 bordeaux mixture plus a half a pound of paris green. Part of the plants were sprayed weekly while others were sprayed bi-weekly on the following dates:—

Sprayed weekly: July 5, 19, 26, August 2, 9, 16, 23, 30, September 6, 14.

Sprayed bi-weekly: July 20, August 2, 17, 30, September 14.

Tip-burn began to appear on the control plots about August 2 but was never as severe as in 1911. On August 30, the difference between the sprayed and unsprayed rows was very noticeable. No other disease prevailed.

Four harvests of the experimental potatoes were made on September 4-5, 18-19, October 2-4, and 30. The results, expressed in terms of a single plant, as well as in calculated bushels per acre, were as follows:—

Strength of spray	Sprayed	Tops lbs.	Large tubers lbs.	Small tubers lbs.	Total tubers lbs.	Large tubers bush.	Small tubers bush.	Total tubers bush.
First harvest; clay loam soil; September 4-5								
5-5-50	Wkly.....	0.41	0.39	0.12	0.51	58.5	17.3	75.8
5-5-50	Bi-Wkly..	0.72	0.73	0.10	0.83	109.4	15.3	124.7
2½-2½-50	Wkly. ...	0.45	0.45	0.08	0.53	67.8	12.2	80.
Check	....	0.52	0.42	0.09	0.51	63.6	13.5	77.1
First harvest; sandy loam soil; September 4-5								
5-5-50	Wkly.....	0.98	0.99	0.10	1.09	148.5	14.3	162.8
5-5-50	Bi-Wkly..	0.56	0.68	0.11	0.79	102.	17.	119.
2½-2½-50	Wkly....	0.89	0.94	0.11	1.05	141.	15.9	156.9
Check	....	0.43	0.55	0.10	0.65	82.5	14.4	96.9
Second harvest; clay loam soil; September 18-19								
5-5-50	Wkly.....	0.88	1.16	0.15	1.31	174.	22.4	196.8
5-5-50	Bi-Wkly.	1.12	1.55	0.10	1.65	232.5	15.	247.5
2½-2½-50	Wkly....	0.88	1.11	0.15	1.26	166.5	21.8	188.3
Check	....	0.61	0.75	0.11	0.86	112.5	16.8	129.3
Second harvest; sandy loam soil; September 18-19								
5-5-50	Wkly.....	1.06	1.24	0.10	1.34	186.	14.7	200.7
5-5-50	Bi-Wkly..	1.27	1.81	0.10	1.91	271.5	15.3	286.8
2½-2½-50	Wkly.....	1.21	1.57	0.11	1.68	235.5	16.8	252.3
Check	....	0.79	1.17	0.12	1.29	175.5	17.3	192.8
Third harvest; clay loam soil; October 2-4								
5-5-50	Wkly.....	0.67	2.29	0.14	2.43	343.5	21.6	365.1
5-5-50	Bi-Wkly..	0.78	1.90	0.11	2.01	285.	16.2	301.2
2½-2½-50	Wkly.....	0.91	2.94	0.16	3.10	441.	23.5	464.5
Check	....	0.38	1.34	0.13	1.47	201.	20.	221.
Third harvest; sandy loam soil; October 2-4								
5-5-50	Wkly.....	0.62	1.80	0.15	1.95	270.	22.1	292.1
5-5-50	Bi-Wkly..	0.62	1.19	0.12	1.31	178.5	18.2	196.7
2½-2½-50	Wkly....	0.73	1.97	0.12	2.09	295.6	18.6	314.1
Check	....	0.58	1.43	0.11	1.54	213.8	16.5	230.3
Fourth harvest; clay loam soil; October 30								
5-5-50	Wkly.....	Dead	2.34	0.23	2.57	351.	34.6	385.6
5-5-50	Wkly.....	Dead	2.50	0.15	2.65	375.	22.5	397.5
2½-2½-50	Bi-Wkly..	Dead	2.39	0.17	2.56	358.	32.6	390.6
Cop. iron sul.	Wkly..	Dead	2.66	0.10	2.26	324.	15.5	339.5
Check	....	Dead	1.97	0.16	2.13	295.5	24.3	319.8
Fourth harvest; sandy loam soil; October 30								
5-5-50	Wkly.....	Dead	1.90	0.15	2.05	285.	23.	308.
5-5-50	Bi-Wkly...	Dead	...	...	...	...	...	....
2½-2½-50	Wkly.	Dead	1.88	0.12	2.00	282.	19.3	301.3
Check	....	Dead	1.59	0.09	1.68	225.	13.2	238.2

The check rows did not yield as many bushels as the average of the bordeauxed ones and the foliage is also slightly lighter in weight on them than it is on that from the sprayed plants. This increase of tubers and foliage of the sprayed plants is much more noticeable in the rows grown on the sandy loam. The same differences are discernible in the second and third harvests with a single exception; the tops of the control plants are lighter and the yield in tubers is less than is the case on the sprayed plots. The results secured at the fourth and final harvest seem to warrant the following statements as applicable to the 1912 experimental conditions.

1. Spraying with bordeaux mixture was equally well worth while on heavy clay loam and on sandy soils although somewhat better results were obtained on the latter type.

2. The amount of copper sulphate and lime used did not appear to be important, providing the mixture was fairly strong. A little difference appeared in favor of the 5-5-50 combination over the 2½-2½-50.

3. Frequent and early sprayings did not seem favorably to affect the yield of tubers. Some of the plants were sprayed ten times; but they produced little or no larger crops than did those sprayed less often.

SPRAYING POTATOES AT BONN, GERMANY, 1912

During the summer of 1912, the writer had an opportunity to make spraying tests and observations on potatoes at Bonn, Germany. He desires at this point to express his appreciation of the courtesy of Professor Max Koernicke of the Poppelsdorf Agricultural Akademie, of Herr Böddinghaus of Bonn and of Herr Weinlich of Vilich. To Professor Koernicke especially are his hearty thanks due for efforts in his behalf.

This region of Germany, while not so well known as a potato growing country as is that around Berlin, produces this crop in great abundance, both in the Rhine valley itself and on the low foothills which lie along the western bank. The soil presents many variations within a small region, for that in the valley is sandy and light while that on the hills is more inclined to be a heavy loam or clay. It was with the intention of testing soil effects on spraying that the trials were made on land in these two localities.

The sprayings on the higher lands were made on the estate of Herr Otto Böddinghaus, the Melb Tal farm, about a mile from Bonn. Two

trials were made here; one on a small field of early potatoes, Paulsen's Julie, located near the Hof and the other on a larger field of late potatoes, Industrie, about a half mile from the Hof. The two varieties were those commonly planted in the country around Bonn and were recommended as being highly resistant to the leaf roll disease.

The land on either plot was a heavy clay loam admirably adapted to potato growing. The spraying operations in the valley of the Rhine were made on the farm of Herr Weinlich, Hof Lede, located near Vilich near Beul across the Rhine and about four miles distant from Bonn. The soil was as light and sandy as that around Burlington, but was considered excellent for potato culture. The variety planted was Industrie.

The spraying was done with a 5-5-50 bordeaux or with "Cucasa," applied by means of a knapsack spray pump, in sufficient quantities thoroughly to cover the foliage. The crops were planted in early April and were far enough advanced by the middle of June to begin the spraying. The applications were made on the following dates:—

Melb Tal farm; on Paulsen's Julie (early variety) June 15, July 1, July 16.

Melb Tal farm; on Industrie (late variety) June 15, July 1, July 16, Aug. 14.

Hof Lede farm; on Industrie (late variety) June 19, July 3, July 17, Aug. 20.

Two hundred plants on each of these plots were sprayed with bordeaux mixture, two hundred with "Cucasa," and two hundred were left as a control. "Cucasa" is a proprietary, clear blue, copper sulphate-calcium carbonate-cane sugar solution of German origin. Trials of this material were made at this Station in 1911, as set forth in bulletin 162. As there are no Colorado potato beetles in Germany, it was unnecessary to use arsenicals on the control rows. The plan of spraying, repeated twice on each of the three plots, provided for 54 plants each treated with bordeaux, two similar rows sprayed with Cucasa and two left untreated.

The early potatoes at the Melb Tal farm were harvested on August 15. At this time the foliage of the bordeauxed plants seemed still to be somewhat greener than that of the unsprayed, the estimate of green foliage for the former being 25-30 percent, for the latter, 10-15 percent. Unfortunately through a mistake two of the control rows were dug before August 15 and no record could be obtained of them. The first two rows sprayed with bordeaux were situated so near a tree that

the end of the rows were shaded after about four o'clock in the afternoon (see Von Oven's work, 33) and consequently the second two rows thus sprayed represent the conditions more fairly. The harvest of the late potatoes at the Melb Tal farm occurred on September 28. It was estimated that about 90 percent of the foliage was dead on the sprayed plants as compared with about 95 percent on the unsprayed. The stalks still were green but the foliage had been killed by a heavy frost about a week before. This early frost was unfortunate, in that it afforded the bordeauxed plants no lengthened season wherein to finish their work, while on the other hand it was fortunate in that it gave an opportunity to show any effect the mixture might have produced due to causes other than lengthened life. No disease of any sort appeared during the growing season; and the tubers were nearly free from scab.

The late potatoes at the Hof Lede farm in the Rhine valley were harvested on October 1. The plants were quite dead with the exception of a few green stems, these being most noticeable in the sprayed rows. The laboratory helper had visited the field about two weeks before this date, before the plants had been affected by the frost, and reported that at that time the sprayed rows still displayed foliage enough readily to distinguish them from the unsprayed areas. The yield here was less than on the Melb Tal plots, but the tubers were large and clean. No disease had appeared in the fields during the growing season.

The following table exhibits the yields, two rows of fifty plants each being harvested in each case. The first two harvests were made on Melb Tal farm on August 15 and September 28, the latter on Hof Lede farm on Oct. 1.

	Paulsen's Julie			Industrie			Industrie		
	Large tubers lbs.	Small tubers lbs.	Total yield lbs.	Large tubers lbs.	Small tubers lbs.	Total yield lbs.	Large tubers lbs.	Small tubers lbs.	Total yield lbs.
Bordeaux ...	136.5	15.5	152.	207.	10.5	217.5	69.	15.5	84.5
"Cucasa"	158.7	19.5	178.2	202.7	10.	212.7	85.5	16.	101.5
Check	178.	16.	194.	192.	15.	207.	81.	17.	98.
Bordeaux ...	159.5	14.8	174.3	187.	9.	196.	78.	18.	96.
"Cucasa"	157.7	17.6	175.3	192.	7.2	199.2	71.	17.	88.
Check	....	...	....	190.5	7.8	198.3	71.	21.	92.
Bordeaux ...	296.	30.3	326.3	394.	19.5	413.5	147.	33.5	180.5
"Cucasa" ...	316.4	37.1	353.5	394.7	17.2	411.9	156.5	33.	189.5
Check	....	...	369.3	382.5	22.8	405.3	152.	38.	190.

In addition to these field trials, a small experiment on potatoes and beans was conducted in the Economic Botanical Garden of the Institute. The potatoes of the Industrie variety, like those in the field, showed no effect which properly may be said to be due to the spraying;

and similar results were secured with the beans. These potatoes were sprayed four times, June 15, June 26, July 16, and August 20. They were harvested on October 7.

	Large tubers	Small tubers	Total tubers	Total tubers per plant
Control, 38 plants	76.5 pounds	13 pounds	89.5 pounds	2.36 pounds
Bordeaux, 37 plants	84. pounds	11 pounds	95. pounds	2.56 pounds

The general results of these experiments indicate that under German climatic conditions spraying either potatoes or beans has little effect upon the plants or upon their yields. The observed differences are too slight to be of consequence in field experimentation and doubtless are due to variations in soil and individual plants. The marked increase obtained under conditions prevailing in America is conspicuous by its absence. Favorable results might have been obtained had the heavy frost of late September held off; but this is very problematical. There certainly would have been no very marked increase in the yield of the sprayed over the unsprayed plants, as they were all in nearly the same stage of maturity. Slightly favorable effects could be observed on the sprayed plants grown on the light sandy soil in the Rhine valley, while on the heavier soils of the foot hills the sprayed and unsprayed plants seemed to be about alike. At any rate it is safe to say that if there were any favorable results brought about by the spraying procedure they were due to the lengthened life of the sprayed plants and not to any stimulus carried by the action of the copper in the mixture.

It should be noted here that the truth or falsity of the doctrine of the stimulating effect of bordeaux mixture on the foliage of the potato plant can be better determined in Europe than in America. The Colorado beetle is not a pest, and one can study the question without having to deal with the possible complications which the insecticides applied for its control may introduce.

That spraying potatoes with bordeaux mixture is not a profitable farm procedure in Germany is evidenced by the fact that few of the growers practice spraying. During recent years there has been an almost complete absence of late blight; and there seems to be lacking also to an unusual degree any evidence indicating that the favorable physiological effects often obtained from the use of bordeaux under American conditions are a factor in Germany.

SPRAYING POTATOES IN 1913 AT BURLINGTON

The summer of 1913 was as favorable a season as the preceding four years had been for field experiments on the physiological effect of bordeaux mixture on potatoes, the plants being entirely free from Phytophthora and Alternaria blights. However, the potato beetle and flea beetle were abundant and were very difficult to control. Some of the seed potatoes were very late in arriving at Burlington so that they could not be planted before June 6. This shortened the growing season by at least two weeks and considerably reduced the yields. Green Mountain and Early Rose were used for purposes of comparison with the two German varieties, Industrie and Paulsen's Julie, that had been used the preceding year in the experiments made at Bonn, Germany. The two American varieties were from New York state seed, and the German seed tubers were obtained from Haage & Schmidt through the courtesy of Dr. W. A. Orton of the Bureau of Plant Industry of the United States Department of Agriculture. The three varieties, Green Mountain, Early Rose and Industrie, were sprayed with 5-5-50 bordeaux, about a pound and a half of dry arsenate of lead being added as an insecticide to every fifty gallons. The control plots were sprayed with arsenate of lead and water and occasionally dusted with paris green and lime.

	Pounds per plant			Bushels per acre		
	Large tubers	Small tubers	Total yield	Large tubers	Small tubers	Total yield
Green Mountain,						
Sprayed	1.54	0.23	1.77	231.6	32.7	264.3
Check	1.41	0.14	1.55	211.3	20.7	232.5
Early Rose,						
Sprayed	1.48	0.19	1.67	221.	28.	249.
Check	1.19	0.16	1.35	178.5	24.	202.5
Industrie,						
Sprayed	0.09	0.54	0.63	13.7	80.9	94.6
Check	0.05	0.63	0.68	7.4	94.6	102.

The above figures show that, notwithstanding the late planting, the yield of the American varieties was increased as a result of spraying operations. This difference undoubtedly would have been much greater if the plants had remained green as is usually the case into October. The apparent small decrease on the German variety, Industrie, hardly can be considered as conclusive as only three rows were used, the middle row serving as a check on the two sprayed rows. In an experiment in another part of the field on the German varieties the results were as follows:—

Industrie		Paulsen's Julie	
Sprayed, pounds per plant	Unsprayed, pounds per plant	Sprayed, pounds per plant	Unsprayed, pounds per plant
1.19	1.09	1.29	.96

The difference in Industrie is slightly in favor of the sprayed rows while it is still more favorable in the case of the early variety, Paulsen's Julie, and it seems safe to say that if the latter had been planted at the proper time, it would have shown a much larger gain. The results on the variety Industrie are to be regarded as inconclusive and the tests were repeated in 1914. The small yield secured from these German varieties is further indicative that they are adapted to a longer growing season than that found in this latitude.

Further trials were conducted on another field of Green Mountain potatoes in which the check rows were sprayed twice and dusted twice with arsenate of lead alone, whereas the other plants were sprayed with various strengths of bordeaux:—5-5-50, 2½-2½-50 and 1¼-1¼-50. Even though the greatest of care was used, the flea beetles did more damage to the foliage of the check plots than to that of the sprayed ones. The plots were sprayed July 12, July 22, August 7 and August 21. A fifth spraying would have been made during the following month, but on September 12 a heavy frost killed the plants.

Green Mountain	Pounds per plant: Large tubers	Small tubers	Total yield	Bushels per acre: Large tubers	Small tubers	Total yield
1¼-1¼-50	2.03	0.09	2.12	304.	13.5	317.5
2½-2½-50	2.17	0.06	2.23	325.4	8.5	333.9
5-5-50	2.18	0.08	2.26	326.2	12.1	338.5
Check	1.53	0.15	1.68	229.5	22.	251.5

SPRAYING POTATOES IN 1914

Two objects were kept in mind in the experiments conducted during the summer of 1914:—(1) to repeat the trials of the effect of sprays on the German varieties of potatoes used during 1912 and 1913, and (2) to determine as far as possible the effect of the bordeaux mixture on a number both of early and late American varieties. The land chosen for this work was very unsuitable in view of the season, for the soil was quite light and sandy. It was the same plot that had been used during the summer of 1909; but the rainfall during that season was not only greater but it was better distributed than during 1914. A supplementary experiment was conducted on an adjacent piece of land, mostly a heavier soil, which yielded somewhat better results. The crop was planted on May 20. A long spell of dry weather then intervened

which prevented much growth during late May and most of June. The first appreciable rain fell June 19. It is doubtful if any germination occurred during these 30 days, since the temperature continued above normal and the soil was like dry sand. Indeed many of the smaller cuttings dried up and never germinated. Others apparently were infected by the Fusarium fungus and, during August, developed the Fusarium wilt. In all, hardly half a stand of normal plants was obtained. The wilt disease was largely confined to an early variety known as the Luther Putnam, bought in the market in Burlington. The results from most of the plots planted with this variety were discarded as being unreliable. Scattering plants affected by the same malady were found in other plots, but as it was felt that the final result would not be influenced materially by this fact, only the badly affected specimens were removed.

PLAN OF POTATO SPRAYING 1914

Variety	Number of rows	Number of plants	Sprayed or unsprayed	Weight of salable tubers	Weight of small tubers	Total weight of tubers
Green Mountain		37	Unsprayed	61.6	3.8	65.4
		52	Sprayed	79.5	7.4	86.9
Luther Putnam		62	Unsprayed	52.6	8.2	60.8
		64	Sprayed	62.1	7.2	69.3
Industrie		26	Unsprayed	15.5	9.7	25.2
		21	Sprayed	20.9	7.4	28.3
Paulsen's Julie		53	Unsprayed	40.7	13.8	54.5
		53	Sprayed	41.	16.	57.
Early Rose		56	Unsprayed	43.6	6.	49.6
		60	Sprayed	54.4	6.6	61.
Green Mountain		61	Unsprayed	63.5	11.2	74.7
		70	Sprayed	86.2	10.4	96.6

Luther Putnam		Unsprayed			
	Not used (too much Fusarium wilt).				
		Sprayed			
Industrie	59	Unsprayed	26.3	18.3	44.6
	45	Sprayed	38.8	13.5	52.3
Paulsen's Julie	58	Unsprayed	27.7	16.3	41.
	60	Sprayed	36.6	15.	51.6
Green Mountain	74	Unsprayed	54.4	9.2	63.6
	70	Sprayed	73.3	4.7	78.
Paulsen's Julie	50	Unsprayed	15.3	12.5	27.8
	48	Sprayed	33.1	14.	46.1
Green Mountain	67	Unsprayed	57.	8.6	65.6
	71	Sprayed	69.2	13.6	82.8
Luther Putnam		Unsprayed			
	Not used (too much Fusarium wilt).				
		Sprayed			
Green Mountain	119	Unsprayed	71.1	14.	85.1
	119	Sprayed	77.4	17.1	94.5
Green Mountain	75	Unsprayed	68.5	9.6	78.1
	84	Sprayed	84.8	13.3	98.1
Green Mountain	96	Unsprayed	92.6	18.7	111.3
	107	Sprayed	122.3	22.1	144.4

Great difficulty always has been experienced in obtaining adjacent plots of land in the vicinity of Burlington with soil nearly enough alike to make the results obtained from them comparable. Soils located only a few yards apart often are radically different. In order to minimize as much as possible the effect of this variant, the sprayed

and unsprayed rows were alternated and, as a rule, the varieties were planted in several portions of the field. The sub-joined plan shows this alternation but in a condensed form. All the plots were four rows wide, the two middle rows being sprayed, while the two outer ones were left unsprayed. This plan paralleled sprayed and unsprayed rows, thus facilitating the work. No space existed between the plots other than that between the rows.

The plots were sprayed on July 14, July 25, August 7, August 22 and September 12, using 5-5-50 bordeaux to which 1.5 pounds of dry arsenate of lead had been added. The control rows were sprayed with an arsenate of lead solution. Some of the plants had to be dusted occasionally with paris green and lime because the arsenate of lead did not at all times sufficiently repress the numerous Colorado beetles.

Observations were made occasionally on the progress of tip-burn. The delayed germination seemed to retard the onset of this ever present malady. Its effect was not marked before late August or early September, although the early varieties such as the Putnam, Early Rose and Paulsen's Julie were attacked in mid-summer.

It should be remarked, however, that the presence of the wilt disease on some of the plants made accurate estimation difficult. Not over five percent of the leaves of the Green Mountain plants were tip-burned by August 15. This observation seems to indicate that tip-burn is a disease which is more likely to appear at certain stages in the maturity of the plant, when the tubers have attained a size large enough to serve as a drain on the energies of the plant on which they are growing, than a malady which occurs at a definite date. At no time during the summer did the late potatoes on the experimental plots suffer as much from tip-burn as they did during preceding seasons. On adjacent supplementary plots, largely Green Mountain with a few Early Rose, tip-burn appeared at a much earlier date and was much more severe. They were located partly on very low ground, a heavy clay loam, and partly on higher ground with a very light sandy soil, similar to that on which the experiment plots were placed. The difference in the amount and appearance of tip-burn on the two ends of the rows was as marked as the difference in the soils. The succulent plants on the damp soil were yellowed and showed an unhealthy condition and incipient tip-burn on July 25, although but little of the disease then actually was present. The plants growing on the higher ground were normal at this time, both as to the appearance of the foliage and the

absence of tip-burn, although their leaves were thinner and less succulent and their stalks formed a looser growing plant. Tip-burn appeared in abundance early in August on the lower level, and by August 22 at least half the leaves on the unsprayed area were lost, although not more than a fourth had died on the higher ground. The difference between the sprayed and unsprayed plants growing on the heavier soil was much greater than upon the lighter soil. Spraying saved at least half of the plants in the parts of the field worst affected.

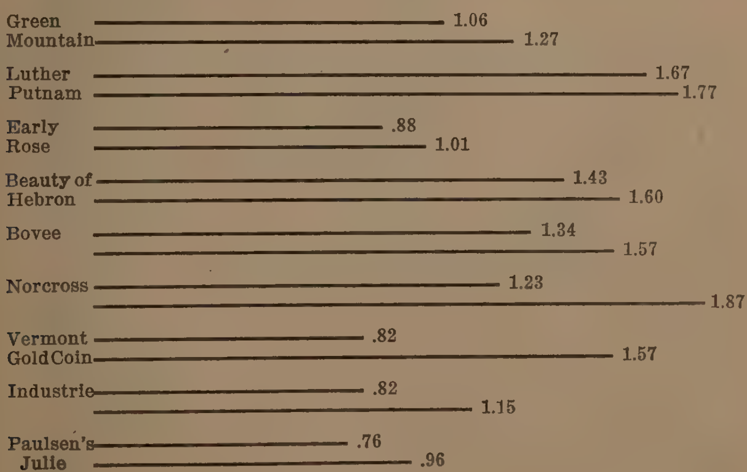
The crop was harvested in October. The yields, together with the plan of the field, are shown in the accompanying table. The results have been summarized for the sprayed and unsprayed plants of the varieties in the table. The yields in bushels per acre were as follows:

	Sprayed	Unsprayed
Green Mountain	192.	159.
Luther Putnam	250.5	265.5
Early Rose	151.5	132.
Beauty of Hebron	240.	214.5
Norcross	280.	184.
Bovee	235.5	201.
Vermont Gold Coin	216.	123.
Industrie	172.5	123.
Paulsen's Julie	144.	116.

The same results, expressed as pounds per plant, are diagrammatically set forth as follows:—

Yields in Pounds per Plant, 1914

(In each case the upper line represents the unsprayed and the lower line the sprayed plant).

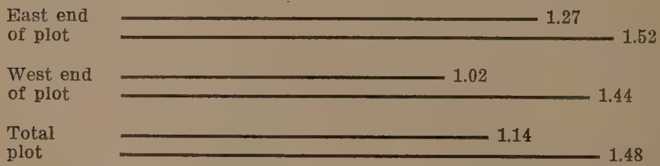


Spraying had a favorable effect in all instances, except with Luther Putnam. In general the late potatoes were advantaged more than the early varieties, but even the early German variety, Julie, was benefited. The very light yields of Industrie and Julie were foreseen and are quite in accord with the experience of other investigators of potato problems who have introduced German varieties into this country, as is evidenced for example in bulletin 179 of this Station, pages 181-183. They are not acclimated to our short, hot summers and do not yield well. The early American varieties show less difference between the yields of the sprayed and unsprayed plants than do the later ones because they die before the effects of the bordeaux become apparent.

The yields from the supplementary plots emphasized the fact that the presence or relative absence of tip-burn account for the differences in yields on the sprayed and unsprayed plants. The earlier it appears and the more severe its attacks, the less the yield. The bordeaux spray retards its onset. The variation in the amount of tip-burn on the two ends of the field was reflected in their potato harvests. The average unsprayed plant on the heavy moist soil died young and produced only 1.02 pounds of tubers, while that on the higher land yielded 1.27 pounds. The differences between the yields of the average sprayed and unsprayed plants on the low land was 0.42 pounds; but on the high land it was only 0.25 pounds.

YIELDS FROM THE SUPPLEMENTARY SPRAYING (pounds per plant), 1915

(In each case the upper line represents the unsprayed and the lower line the sprayed plant).



The west end of the plot was lower and the soil much heavier than that of the east end.

GREENHOUSE AND POT SPRAYING TRIALS IN 1914

The outcome of the experimental trials made in 1914 in and immediately back of the plant pathology greenhouse was most satisfactory. They furnished convincing evidence that the effect of the

bordeaux spray is conditioned upon climatic considerations. Larger pots and deeper benches might have been used to advantage, but on the whole the trials were a success.

Thirty-two plants were grown in the greenhouse. Medium sized Early Rose tubers were cut into pieces weighing from 35 to 36 grams. These were dried over night and planted April 17. Sixteen, all that were placed in one bed, rotted and were replanted on May 4 by similar pieces. Sixteen of the plants were sprayed with 5-5-50 bordeaux, alternate ones being chosen, on May 21, June 10, June 25, July 23, and August 25. The spray was applied as often as it washed off to any considerable extent. The plants were kept well watered and the greenhouse was well ventilated both night and day. Both the sprayed and unsprayed plants were examined for tip-burn, but not the slightest difference could be detected in the foliage. About a third of the tops were still green on October 6, but since the tubers were beginning to sprout it was thought best to harvest them.

GREENHOUSE EXPERIMENT

East Bench				West Bench			
Sprayed or unsprayed	Number of tubers	Weight of tubers grams	Average Weight grams	Sprayed or unsprayed	Number of tubers	Weight of tubers grams	Average Weight grams
Sprayed	14	793	56.6	Unsprayed	6	170	28.3
Sprayed	13	585	45.0	Unsprayed	17	708	41.6
Unsprayed	11	920	83.6	Sprayed	13	522	40.1
Unsprayed	12	694	57.9	Sprayed	9	433	38.1
Sprayed	14	915	65.3	Unsprayed	11	397	36.1
Sprayed	11	538	49.0	Unsprayed	2	347	173.5
Unsprayed	10	1028	102.8	Sprayed	7	366	52.3
Unsprayed	7	412	58.8	Sprayed	5	521	104.2
Sprayed	14	688	49.1	Unsprayed ...	6	342	57.0
Sprayed	13	557	42.6	Unsprayed	7	700	100.0
Unsprayed	15	1055	70.3	Sprayed	5	313	62.6
Unsprayed	7	617	88.1	Sprayed	9	507	56.3
Sprayed	8	746	93.2	Unsprayed	10	373	37.3
Sprayed	16	787	47.9	Unsprayed	9	690	76.6
Unsprayed	11	982	89.2	Sprayed	8	406	50.7
Unsprayed	7	746	106.5	Sprayed	8	660	82.5

	Tubers	Weight grams	Average weight tubers per plant in grams	Average weight tubers, grams
Unsprayed	148	10,181	636.3	68.7
Sprayed	167	9,337	583.5	55.9

Out-of-door pot experiments were carried on simultaneously with the greenhouse trials. Twenty zinc pots containing each about 6,000 grams of soil to which about 20 grams of potato fertilizer (4 percent nitrogen, 15 percent total phosphoric acid, 6 percent potash) had been added were planted on May 4 with Early Rose cuttings, each weighing

from 36 to 37 grams. The plants were left in the greenhouse until June 10, when they were removed to the open air and sprayed. They were sprayed also on June 25, August 9, and September 8. The foliage suffered greatly from tip-burn during the fourth week in August, when almost a third of it was thus destroyed. The malady was noticed first in mid-July, during a period of very hot weather. A high temperature during the day seems to be the direct cause of this disease, as has been shown many times. The bordeauxed plants suffered somewhat less than did those that were not sprayed. The soil in the pots was kept thoroughly moist, but painstaking care in this respect did not prevent tip-burn injury both to sprayed and unsprayed foliage. The entire top of the unsprayed plants, both leaf and stalk, was dead by August 15, whereas the sprayed plants still retained about half their foliage and all their stalks, although they were not of a healthy green color. All the unsprayed plants were dead on August 28 when they were harvested. The sprayed plants were harvested three weeks later on September 18, when a few stalks were still somewhat green. The results were as follows:—

POTATO PLANTS IN POTS, IN THE OPEN

	Unsprayed			Sprayed		
	Number of tubers	Weight of tubers	Average weight	Number of tubers	Weight of tubers	Average weight
		grams	grams		grams	grams
	5	302	64.4	9	325	36.1
	8	273	34.1	10	388	38.8
	7	241	34.4	10	349	34.9
	9	285	31.6	8	361	45.1
	9	274	30.4	6	281	46.8
	6	228	38.0	6	353	58.8
	8	277	34.6	7	406	58.0
	12	292	24.3	7	295	42.1
	6	229	38.2	7	334	47.7
	13	397	30.5	3	291	97.0
Total	83	2,798	33.7	73	3,373	46.2

It would appear from the results secured in this greenhouse trial where the soil was absolutely uniform, the climatic conditions fairly under control and the seed of uniform size:

1. That the yields were less from sprayed than from unsprayed plants.

2. That the tubers from the sprayed plants were smaller in size than from the unsprayed ones.

The potted sprayed plants grown outdoors increased in weight as compared with the unsprayed ones quite as much as usually is shown in our field trials. In both cases the plants were provided with the

same soil and an abundance of water. The difference in the outcome between the indoor and outdoor plants was clearly due to effects of the flea beetle and the tip-burn. On the indoor plants these troubles practically were absent; on the outdoor plants, they were very much in evidence and materially shortened the life of the unsprayed plants.

SPRAYING POTATOES, FIELD TRIALS, IN 1915

The potatoes used for the field sprayings were Irish Cobblers grown back of the farm buildings and planted late in May. Not more than half of the seed germinated because of Rhizoctonia. Many of the plants which grew were severely attacked by this disease and the field presented a sorry appearance.

The usual mixtures were applied on July 20, July 29, August 12 and August 24, the first spraying being delayed in the hope that more of the plants would grow.

The difference between the sprayed and unsprayed plants was very marked on August 30, the unsprayed plants being half dead while the sprayed ones hardly were touched. The brilliant sunshine of August 27-28 had produced this change. The foliage died rapidly between September 8 and 11 when the weather was hot and the sunshine brilliant. The unsprayed plants were from two-thirds to three-fourths dead at this time while the sprayed tops had lost not more than a third of their foliage. During the following two weeks the plants slowly died and harvest was begun on October 1. A few leaves infected with late blight were found as well as some affected with early blight. The amount of leaf disease of this sort was not of sufficient consequence to affect the results.

The tuber yield per sprayed plant was 2.18 pounds, 0.47 pounds of which were small and 1.72 pounds salable. The tuber yield per unsprayed plant was 2.08 pounds, 0.54 pounds of which were small and 1.54 pounds large.

SPRAYING PLANTS IN THE GREENHOUSE IN 1915

Early Rose tubers were planted on April 30 in parallel beds, the soil of which had been mixed so thoroughly that strict uniformity must have been secured. The seed pieces approximated 60 grams in weight. Ten plants were grown in each bed, and sprayed on June 10, June 17, and June 28. The potatoes were harvested on July 15, when the unsprayed plants were nearly all dead and the sprayed ones

about half dead. For some reason—probably more brilliant sunshine—the crop suffered from tip-burn to a greater extent than during the preceding season. The reduction in the intensity of the sunlight produced by the shelter of the greenhouse was not sufficient to prevent the premature death of the unsprayed leaves. Two further differences are to be noted; the potatoes were planted later in the season and the first spraying was done at a later date. The destruction of the leaf areas was of a somewhat different type from that which occurs in ordinary tip-burn. The browning instead of appearing on the ends and sides of the leaflets seemed to affect them in irregular spots and they succumbed as a whole and not in parts.

As was to be expected, in all cases where the unsprayed plants died before the sprayed ones the yields were relatively less. If the harvest had been delayed, the differences in yield would have been even larger for the reason that half of the sprayed plants were still living in mid-July whereas all the unsprayed ones had died.

RESULTS EXPRESSED AS GRAMS PER PLANT AND TOTALS

Unsprayed	335	365	260	385	425	216	315	410	360	612	3,683
Sprayed	475	425	255	655	370	477	312	265	280	455	3,969

SPRAYING POTATOES IN TILES 1915

Twenty large tiles 12 inches in diameter were filled with carefully mixed soil and manure and planted on May 16, Early Rose cuttings averaging 70 grams each in weight being used. Half of the number were sprayed on June 28, July 15, and August 11 the tops being at all times well covered by the spray mixture. Tip-burn was first noticed on the lower unsprayed leaves on July 25 and was quite marked by July 31. The tubers were harvested on September 10.

Unsprayed: Number of tubers	Unsprayed: Weight grams	Sprayed: Number of tubers	Sprayed: Weight grams
41	1,158	35	1,724
16	1,298	29	1,788
32	1,118	14	1,771
22	1,271	24	1,463
18	947	43	1,320
28	1,160	16	1,618
23	998	22	1,270
14	1,163	32	1,268
21	1,297	22	800
20	1,104	24	1,393
235	11,514	261	14,415

The difference in the yields is about same as usually is shown in field trials and is very similar to that obtained the preceding year. The

tip-burn and flea beetle injury, both of which were severe on the unsprayed foliage, seemed to be the cause of the lessened yield from the untreated plants.

SPRAYING BEANS, 1911

It is not easy to measure with certainty the outcome of spraying with plants other than the potato, for the reason that unlike that plant they possess no storage organ in which practically all the carbohydrates are held. Beans were used for two trials as it was felt that they met the requirements as nearly as any of our commonly cultivated plants. The bean contains a large percentage of protein and the assimilation of the starch into protein may be affected by the presence of the copper salts on the foliage in such a way that the process is rendered more difficult and more starch is consumed in the process. This consideration would have to be borne in mind if the yield in pods or beans is to be taken as a measure of the effect of the spraying on the plant. In spite of this possible complication the bean was used, since no better plant could be found.

Eight rows of bush beans were planted and the alternate rows sprayed in 1911. The beans were gathered on August 15 and 16 when they were about of the right size for shell beans. The pods are included in the weights.

Unsprayed	2,100 plants	38,502 grams beans	18.3 grams per plant
Sprayed	1,990 plants	38,464 grams beans	19.3 grams per plant

Alternate rows of bush beans were sprayed three times in the summer of 1912 in the Economic Botanical Garden at Bonn, Germany.

	Number of plants	Total weight of pods and beans grams	Weight per plant of pods and beans grams	Total weight of shelled beans grams	Weight per plant of shelled beans grams
Unsprayed	77	532	6.9	352	4.57
Sprayed	80	561	7.0	380	4.72

The differences in yield between the sprayed and unsprayed plants are so slight that soil variations easily might have produced them. The bordeaux spraying seems to have had no marked effect, either favorable or unfavorable. Bayer (6) claims to have secured very favorable results on the foliage following the spraying of the bean, but gives no figures showing comparative yields.

SPRAYING ARTICHOKES IN 1913 AND 1914

Eleven 40-foot rows of artichokes were planted June 2, 1913. They were sprayed three times with 5-5-50 bordeaux. The

foliage did not seem to be affected. The plants were killed by the frost and the tubers left in the ground to come up the following spring. The same rows were sprayed twice during the summer of 1914 and the tubers were harvested October 2. The unsprayed rows yielded 324.6 pounds and the sprayed rows 297.3 pounds. Apparently the spraying, if it accomplished anything, tended to injure the crop.

2. Morphological Changes

About twenty-five years ago, it began to be noticed that when grape foliage was sprayed with bordeaux, the leaves were thicker, of a bluish green color and lived longer than when they were left unsprayed. Rumm (36) seems to have been the first to call attention to these differences and his observations have been confirmed, especially on the potato plant, by later investigators such as Frank and Krüger (17), Schander (38), Ewert (16), Zucker (47), Aderhold (1), Harrison (20) and others. These changes undoubtedly are brought about by the absorption of minute quantities of copper into the leaf. This absorbed copper must be very small in quantity, otherwise the leaf would be injured or its physiological functions at least seriously interfered with. Some plants are able to absorb more of this copper without injury than others. The peach, plum, apple, and cherry are relatively susceptible, while spray injury, due to improperly made bordeaux with a consequent absorption of copper, is less frequent on the grape and potato. The copper taken into the growing leaves produces certain morphological changes in their structure. Rumm (36) made numerous measurements of the cells and thickness of the grape leaves, expressed as microns, the following of which will serve as an illustration:—

TWO LEAVES FROM A PLANT IN THE EXPERIMENT HOUSE, SEPT. 12

	Unsprayed microns	Sprayed microns	Increase microns
Thickness of leaf	156.63	172.92	+16.31
Epidermis	15.81	18.38	+ 2.57
Palisade tissue	55.07	61.87	+ 6.80
Parenchyma	85.75	92.69	+ 6.94

TWO LEAVES FROM A PLANT IN THE EXPERIMENT HOUSE, SEPT. 14

	Unsprayed microns	Sprayed microns	Increase or decrease microns
Thickness of leaf	155.02	157.19	+ 2.17
Epidermis	15.48	16.28	+ 0.80
Palisade tissue	53.82	59.18	+ 5.36
Parenchyma	85.72	81.73	— 3.99

The chlorophyl bodies in the palisade tissue of the sprayed leaves were somewhat smaller but more numerous than those in the unsprayed

leaves. The parenchyma cells from the sprayed plants were richer in chlorophyl and contained fewer vacuoles.

Frank and Krüger (17) record no measurements of leaves from sprayed and unsprayed potato plants. Their figures of leaf sections are diagrammatic, made only for the purpose of showing the relative amount of starch in treated and untreated leaves. They have made the observation, however, that the results secured from the potato plant agree with those attained with the grape, as Rumm has found; but the differences in thickness between the sprayed and unsprayed leaves are so small that they almost disappear in the sections. The difference in favor of some bordeauxed leaves is quite marked, while in other cases it is hardly noticeable; but it is never true that the unbordeauxed leaves are thicker than the treated ones.

Zucker (47) made a series of observations on the direct influence of bordeaux mixture on a variety of plants. These were not at all in agreement. *Nicotiana collossea* leaves seemed to be affected as were the grape and potato, but practically no change was wrought as a result of applying bordeaux on the leaf structure of *Ricinus communis, Colens hybrida, Pereskia aculeata, Begonia Lebrina, Eucalyptus tereticornis, Melalenca alba, Tydaea grandiflora, Cestrum roseum, Aralia pentaphylla and Juniperus Bermudiana.*

Lodeman (28) made the statement that spraying with bordeaux increased the thickness of plum leaves from 1.9 to 2.8 percent and of German prune leaves 10.2 per cent. Harrison (20) attempted to confirm his observations and found that plum, pear, peach and quince leaves were slightly thickened, probably because of the increased development of the palisade layer of cells, and that a marked development of chlorophyl granules occurred in the cells.

Schander (38) and Ewert (16) give no data touching the morphological effect wrought on the leaves by the mixture further than to verify the general observations previously made on plants thus treated.

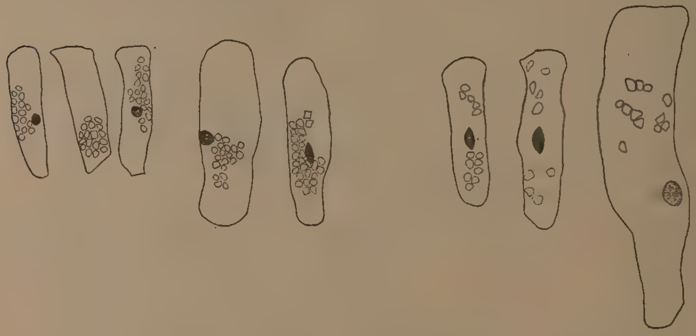
Both Sorauer (39) and von Schrenk (40) have observed the formation of intumescences, the former on potato leaves, the latter on cauliflower, brought about by the action of copper salts on the cells of these plants. Sections of these growths showed them to be composed of parenchyma cells so strongly hypertrophied as to break the epidermis.

So far as the writer knows no careful measurements have been made of potato leaf cells secured from sprayed and unsprayed plants. The following data were derived from such studies of Green Moun-

tain leaves grown in the field. Part of the material was fixed in Flemming's solution, sectioned and stained, while part was preserved in weak formalin and free-hand sections used. Leaflets as nearly alike as possible in area were taken; Nos. 1 and 2 are comparable, Nos. 3 and 4, Nos. 5 and 6 and so on.

		Palisade parenchyma microns	Pulp parenchyma layer microns
1.	Unbordeauxed, cells near margin ...	885 × 216	840
	Unbordeauxed, cells near mid-rib....	858 × 204	990
2.	Bordeauxed, cells near margin	1044 × 222	900
	Bordeauxed, cells near mid-rib.....	876 × 192	1200-1350
3.	Unbordeauxed, cells near margin ..	660 × 210	1050
	Unbordeauxed, cells near mid-rib...	1042 × 228	1050-1200
4.	Bordeauxed, cells near margin.....	1320 × 210	1500
	Bordeauxed, cells near mid-rib.....	1950 × 300	1650-1800
5.	Unbordeauxed, average thickness...	750 to 840	900- 960
6.	Bordeauxed, average thickness......	900 to 1050	1050-1140
7.	Unbordeauxed, average thickness...	1050 to 1200	1050-1200
8.	Bordeauxed, average thickness.....	1050 to 1200	1350-1500
9.	Unbordeauxed, average thickness...	750	900-1050
10.	Bordeauxed, average thickness.....	900	1050-1200
11.	Unbordeauxed, average thickness...	900	1200
12.	Bordeauxed, average thickness.....	1050-1200	1200

It will be noted from these data that the leaves from the bordeauxed plants show in general thicker palisade and pulp parenchymas than do the untreated ones. This hypertrophy is particularly marked in the length of the palisade cells of the leaf (fig. 9). The increase



1 2 3 4

FIG. 9. Palisade cells from unsprayed and sprayed leaves. Nos. 1 and 3, unsprayed; Nos. 2 and 4, sprayed.

in the size of the cells was almost entirely in length but in a few cases where the hypertrophy was very pronounced, lateral bulging also occurred. The result of this stimulus was the production of long, comparatively slender, palisade cells. The morphological changes were less marked in the cells of the pulp. On account of their irregularity

it was impossible to make measurements of the individual cells of this layer, but the thickness of the entire layer usually is somewhat increased. It would be expected naturally that the cells immediately beneath would be affected most by the absorption of minute quantities of copper from the surface of the leaf, but no decided increase in the thickness of the epidermal cells could be observed. The stimulus seems on the other hand not to penetrate into the leaf to a sufficient depth to cause any large increase in the size of the pulp cells. No indications were seen of a dying off of the epidermal cells and a lengthening of the palisade cells into long tube-like growths deficient in chlorophyl, as described by Sorauer (39). The possible explanation for the appearance mentioned by Sorauer is the presence of little uncombined copper in the mixture applied, although it may have been brought about by unfavorable weather conditions, for he notes that these abnormal growths occur also on the unsprayed leaves. He explains this hypertrophy of the palisade cells as due to the diminished powers of assimilation of these plants accompanied by the presence of too much water.

It was difficult to count the number of chlorophyl bodies in the cells, but there seemed to be some increase in their number in the cells derived from the sprayed plants. If the hypertrophy of the cells was very great, however, the individual chloroplasts also were enlarged. The nucleus also was increased in size noticeably in the abnormally large cells.

The real physiological disturbance which produces these unusually large cells is difficult to locate, but an increased turgor is the probable immediate cause. The way in which this turgor is brought about is not clear. Ewert's theory (16) that the presence of copper salts interferes with the removal of the starch during the night, might have a bearing also on the turgidity of the cells from which this starch is removed. The constant presence of glucose, due to its slow removal under these conditions, would tend to raise the osmotic concentrations of the cell sap and probably would tend to increase turgor.

3. Physiological Changes

The morphological effects produced by spraying plants with bordeaux mixture seem to be followed by physiological changes which manifest themselves in the yield of tubers. The cultivated potato, it should be pointed out, is the most perfect starch producing machine that man has found, and consequently is the best plant available for

measuring such effects. The slightest disturbance in any of its ordinary functions may affect either favorably or unfavorably the quantity of the potato tubers produced. The appearance in America of the sprayed plants as compared with those unsprayed is markedly modified as shown by:

(1) The dark green color with increased chlorophyl content.

(2) The thicker leaves and sturdier stalks.

(3) The longer life—ten days to two weeks—of the crop.

All this would lead the observer to expect an increase in the weight of tubers produced by sprayed plants. This expectation has been confirmed without exception by every American investigator who has sprayed potatoes with bordeaux. The longest unbroken record in this country of such sprayings is that of the Vermont station, where work was begun in 1891 and now has been carried on for a quarter-century. During at least a third of these 25 seasons very little disease appeared in the fields. Under such conditions any increase following the use of bordeaux must have been due to some action other than a fungicidal one. The smallest increase due to the spraying during all these years was that obtained in 1906, namely 32 bushels per acre, while as high an increase as 72 bushels per acre is recorded in years when no disease such as late or early blight caused a loss in the foliage or tubers of the unsprayed plants. The New York (Geneva) station has practiced spraying since 1902, and the results it has obtained have paralleled those secured during the same seasons at Vermont (41). These trials were conducted not only at Geneva but at other points throughout New York State, and always with favorable results. An increase of 39 bushels per acre in 1908 from the plots sprayed at Geneva was noted; and 1908 was the sixth year in which neither early nor late blight infestation occurred and in which no potato rot was in evidence. These favorable results are ascribed by the New York botanists to the protection afforded the plants against flea beetle and tipburn. It has been noted repeatedly at both these stations that the increase in yield brought about by the use of bordeaux is in the marketable tubers. The amount of culls usually remains about the same.

Numerous shorter trials have been made in various parts of the United States which corroborate the results of these two long-time experiments. For example Giddings (18) in West Virginia found in 1909 that three applications of bordeaux increased the yield 53.5 percent and that four applications in 1910 augmented it by 39.3 percent.

The unanimous verdict of all American observers and investigators to the effect that spraying the potato crop with bordeaux mixture is worth while and that usually it "pays," is in marked contrast to the point of view expressed by European students of potato culture, based on the results secured in similar experiments. These have been made in almost countless numbers. The fact is that the physiological effect of bordeaux seems to vary in different regions, soils, and years. Kirchner (24) has collected six instances in which the sprayed potatoes yielded a smaller harvest than the unsprayed; four in which at least some of the sprayed varieties yielded a greater weight than the unsprayed, an increase brought about by the lengthened life of the sprayed plants; and five instances in which, for some reason which he does not understand, the sprayed plants yielded more than the unsprayed ones. His own results with bordeaux secured in 1904, 1905 and 1907 are cited below, the yield from the unsprayed crop being expressed as 100 in each case.

1904. *Leo.* Sprayed 3 times with 1 percent strength bordeaux,[1] 97.9 percent; 4 times, 1 percent strength, 92.2 percent; 5 times, 1 percent strength, 92.2 percent; 6 times, 1 percent strength, 93.9 percent.

1905. *Cimbal's Frühe Reichtragende.* Sprayed 3 times with 0.5 percent strength bordeaux, 112.1 percent; 1 percent strength, 121.1 percent; 2 percent strength, 115.4 percent; 3 percent, 109.9 percent.

1907. *Olympia.* Sprayed 4 times with 2 percent strength bordeaux, 64.4 percent (starch content, 68.4 percent, starch content of unsprayed plants being 100).

The sunshine, both in quantity and intensity, was very much below the normal for July and August, 1907, and it is to this factor that Kirchner ascribes the low yield from the sprayed rows. A normal amount of sunlight had occurred during other growing seasons and the results were more nearly those likely to be secured during an average summer. The conflicting results obtained by different investigators are due, therefore, according to Kirchner, partly at least to seasonal differences. The shading produced by bordeaux on the foliage in seasons when all the sunlight available should be utilized, results in a lessened yield, while the foliage is protected from excessive sunlight in summers when it is abundant and strong.

PREVIOUS WORK ON THE PHYSIOLOGICAL EFFECT OF BORDEAUX

It is to be expected of course that a problem such as that of the influence of bordeaux mixture on plants, having both great practical

[1]One percent bordeaux corresponds approximately to the 5-5-50 mixture; 2 percent, to the 10-10-50 mixture and 3 percent in the 15-15-50 mixture.

and scientific interest, should be taken up by a number of investigators. The results which they obtained and the theories based thereon are so divergent that it seems necessary to review the work of each individually.

Rumm (35) found that he was unable to detect spectroscopically the copper lines in the ashes of sprayed leaves which had been carefully washed of all external traces of bordeaux mixture. Hence he held that the effect of the copper salts in the mixture was not due to minute quantities which had been absorbed, but to chemotactic influences due to the presence and intimate contact of the copper salts with the cell walls. This stimulus of the copper salts acts on the life functions of a plant in a similar manner as does light and gravity, and expresses itself in an increased production of chlorophyl. This unjustifiable extension of Pfeffer's theory as to the production of chemotactic influence in the plant has not been regarded with favor by any succeeding investigator with the exception of Zucker (47). Rumm was convinced that the application of bordeaux had a favorable effect on sprayed plants, due largely to the lowering of transpiration. He maintained that the copper-lime combination stimulated those forces which hold the water in the living cells and that whether the chlorophyl content of the leaves was made larger or smaller as a result of the use of bordeaux was a matter of secondary importance.

Frank and Krüger (17) were able to state that spraying potatoes with copper increased the yield in those years in which the plants were free from Phytophthora infestation. The harvest from sprayed and unsprayed plants in 1893, a very dry season in Germany, stated as kilograms per plant, was as follows:—

	Early Rose	Fürst von Lippe
Unsprayed	0.175	0.168
Sprayed with lime	0.178	0.217
Sprayed with weak bordeaux	0.180	0.169
Sprayed with strong bordeaux	0.189	0.175

The actual presence, shown both macroscopically and microscopically, of more starch in the sprayed leaves than in the unsprayed ones was demonstrated by means of the Sach's iodin test on leaves from which the chlorophyl had been removed by alcohol. Sections of sprayed leaves showed the chlorophyl bodies filled with large starch grains in contrast with the small and less numerous ones seen in unsprayed cells. The favorable influence of bordeaux on potato plants, as evidenced in an increased yield of tubers, was held by these investigators to be a result of increased transpiration in the sprayed plants,

and of their lengthened life. No explanation is offered as to the cause of the increase of transpiration following the application of copper salts to the foliage; although these authors suggest that the key is to be sought in the stronger assimilation of such leaves, an increased formation of material going hand in hand with an increased use of water. No theory of any sort is advanced as to the lengthened life of the sprayed plants, but the trials showed that it was due to the copper for the reason that the leaves sprayed with lime alone died as promptly as did those left unsprayed.

Aderhold (1) stated it as his opinion that the favorable results received with bordeaux were not due to the lime or copper sulphate, but to the traces of ferrous sulphate that are always present as an impurity in copper sulphate, three commercial samples of which on examination were found to carry .052, .034 and .052 percents of ferrous sulphate. The favorable action of iron salts in chlorophyl formation long has been recognized in the treatment of chlorosis in grape vines. The results of experiments on beans sprayed in part with bordeaux made from chemically pure copper sulphate and in part with that made from the commercial salt, convinced him that iron played an important role in the favorable effect of the mixture.

Zucker's contribution (37) was an extension of Rumm's and of Frank and Krüger's work to a large number of plants, the majority of which were greenhouse species. Plants sprayed with bordeaux showed greater resistance to etiolation than did unsprayed ones; their chlorophyl contents were increased, a stimulated power of assimilation was evident, and the shoot lived longer. Without a single exception among the plants used in these experiments, those sprayed with bordeaux transpired more than did either the unsprayed or those sprayed with lime alone. Zucker's explanation was to the effect that some sort of electrical force acted between the salt solutions in the cell sap.

Bayer (6) made use of bordeaux and various other compounds of copper in culture solutions in an endeavor to ascertain what effect these salts had on the root systems and foliage when taken into the plant body in connection with the nutrient materials and water. It is not necessary to discuss this phase of the absorption of copper compounds here, as probably it is quite different in its effect from a similar absorption through the foliage. Bayer was unable to confirm Frank's, Krüger's and Zucker's observations that sprayed plants transpire more than do unsprayed plants. In two trials with potatoes he found transpiration to be slightly increased where spraying had been

done, but the reverse was found to be true with bean and pea stalks. He felt that the lessened transpiration in these plants was caused by the lengthening of the palisade cell spaces in the pulp parenchyma; that long palisade cells expose less surface to the exterior and, therefore, transpire less than do shorter ones possessing more end surface under the epidermis.

Schander (38) in a very long paper on this general subject supports strongly the stimulus theory. He holds that the copper penetrates the leaf in small quantities, probably less than one part in a hundred million, and there produces changes in the assimilation and transpiration of the foliage. He disregards the influence of the lime and of the iron in the copper sulphate. He ascribes the favorable results to the shading produced by the bordeaux on the upper surface of the leaves. The assimilatory tissues lying beneath the epidermis are protected in this manner from the injurious excess of sunlight and the amount of carbon dioxid is fixed in larger quantity than it would be if the plant were exposed to the full strength of the light. Increased assimilation from the use of the mixture is obtained in very dry seasons such as 1892 (Rumm), 1893 (Frank and Krüger), 1896 (Aderhold), while unfavorable results may happen in summers that are very wet or cloudy, as was 1891 in part, when Sorauer carried on his field experiments. He states that the difference in color between sprayed and unsprayed plants goes back to the same cause; that shaded plants always are a darker green than those exposed to the full sunlight. He finds, as do others whose work is cited, that transpiration is diminished about 10 percent in sprayed plants, but he believes that this is due to the slight lowering of the temperature resulting from the shading, a difference of 0.1 to 0.5 degrees C.

Ewert (16) attempted to solve the problem of the effect of bordeaux on plants by means of pot cultures grown under carefully regulated conditions of soil and temperature. The zinc vessels used as pots each contained the same quantity and quality of soil; tubers of the same size were planted in them; the soil was maintained at a definite saturation; and the plants were carefully rolled in and out of a shelter house at night and during rains. Ewert intended to verify or disprove the Schander theory that the favorable action of the bordeaux was due to the shading which the mixture produced on the leaves. A part of the plants, therefore, were grown under green or white cloth or white gauze screens. Field experiments also were made under similar conditions. The results obtained indicated that in all

instances the spraying with bordeaux, the shading with cloth, and the combination of the two, diminished the yield of tubers. The amount of water used on the several pots was recorded. Those containing sprayed plants lost by transpiration less than those containing the untreated ones. The respiration of the sprayed plants was slightly lowered by the treatment; this was never marked, but was very constant.

Ewert's most original contribution to this question was his discovery that the bordeauxed plants contained more starch in the morning in the leaves than did those which had not been thus treated; not because they were making more starch but because they were unable to get rid of it as rapidly as do the unsprayed ones. This results in a heaping up of starch in the chlorophyl bodies and the impression is that their assimilation is greater than is that of those derived from untreated leaves, a point of view accepted by every other investigator who has tested them according to the Sach's method. Ewert has pointed out that even after 16 hours in the dark the starch is still held in patches in the sprayed halves of leaves taken from the same plant. The retarding of the removal of the starch is due to the action of the copper on the diastase. By means of experiments made outside the plant, Ewert (15) was able to show that even in a concentration of one part of copper sulphate in thirty million of water, the action of small quantities of diastase on starch was prevented. This small percentage of copper easily might be absorbed by the plant, thus preventing the removal of the starch at night. This work was very carefully done and the conclusions as to shading and the effect of copper on the diastase of the leaves are thoroughly worthy of consideration.

(a) WHICH CHEMICAL CONSTITUENT STIMULATES THE POTATO PLANT?

Aderhold's (1) contention that the iron sulphate nearly always present in commercial copper sulphate is the cause of the brilliant green color of sprayed plants, had already been disproved by Ewert (16) under European conditions, but it was thought well to devote one season's field trials to a test of this matter, especially as this country is the only one in which consistently favorable results are obtained when bordeaux is applied. Plots of Green Mountain potatoes were sprayed in 1909 with the ordinary 5-5-50 bordeaux formula, with bordeaux containing iron sulphate, (2 pounds copper sulphate, 4 pounds iron sulphate, 6 pounds lime, 50 gallons water) and with iron sulphate and lime, (5 pounds iron sulphate, 5 pounds lime, 50 gallons water).

Control plots were dusted with paris green and lime. The foliage of the plants on the two plots which were sprayed with the compounds containing copper were the only ones which showed the dark bluish green appearance characteristic of plants sprayed with copper compounds. The plants sprayed with iron sulphate and lime were as yellowish green in appearance as were the controls. The yield of tubers in bushels per acre confirmed these observations on the foliage:—

Treatment	Marketable tubers bushels per acre	Small tubers bushels per acre	Total yields bushels per acre
Bordeaux (5-5-50)	192	51	243
Bordeaux-iron sulphate (2-4-6-50)	184	51	235
Iron sulphate and lime (5-5-50).......	117	51	168
Control (paris green and lime)	138	50	188

The yield on the plots sprayed with iron sulphate and lime was less than it was on the control plots. These results seemed to prove conclusively that in this country, as Ewert showed was the case in Germany, the copper is what changes the appearance of the foliage and augments the tuber yields. Hence no further experiments along this line were attempted.

(b) DOES THE COPPER PENETRATE INTO THE PLANT STRUCTURE?

In view of the fact that negative results were obtained by Frank and Krüger (17) and by Rumm (35) when applying spectroscopic analysis to the ash of the foliage of sprayed plants, it would appear that if the copper really does enter the leaf it must be in very minute amounts. However, Ewert (15) was able to demonstrate by its action on the diastase that copper was present in the sprayed leaf in minute traces. Indeed there now seems to be no reasonable doubt that it can be absorbed by the leaves. Pickering (34) found that nine apple leaves, with a weight of 9.7 grams and an area of 60 square inches, reduced in 24 hours the amount of copper in 275 cc. of .001 solution of copper to .0007, and after three days, to .0004. He was also able to demonstrate, chemically, the presence of copper in sprayed apple and orange leaves.

The effect of copper on the keeping qualities of chlorophyl in solution was first noted in the Vermont laboratory in some sprayed and unsprayed plants preserved during the summer for a comparative determination of the amount of chlorophyl. The alcohol containing the sprayed plants retained a brilliant dark green hue, while that containing the unsprayed tops was greenish brown. Some of the copper salts from the exterior of the sprayed plants were still clinging to

them and aided in their preservation. Alcoholic extracts of potato chlorophyl were made and to them was added 0.1, 0.01 and 0.001 percents of copper sulphate. These solutions, together with a check tube of chlorophyl, then were exposed direct to the sunlight for about an hour. The chlorophyl in the check tube became a brownish green, the liquid in the tube containing the 0.1 percent was unchanged whereas those in the 0.01 percent and 0.001 percent tubes were both much greener than the solution in the tube containing no copper salts. Two plants were placed over night, upside down, in a jar containing 0.1 percent solution of copper sulphate, while two were placed in tap water. The former lot were more flaccid in the morning than the latter. Solutions of chlorophyl made from these plants showed the same differences in their resistance to the action of sunlight that was shown in the previous experiment, the plant having taken up enough copper during the night to make its resistance to the destructive action of the sunlight very marked. Plants treated in a similar manner were dried in the direct sunlight. The plants from the copper sulphate solution remained bright green even when kept for weeks in the sunlight, while the check plants turned a greenish brown in drying. The bordeaux mixture was washed from sprayed potato plants grown in the greenhouse by means of weak acid and water until no trace of copper showed in the washings. An alcoholic solution of chlorophyl was then made by boiling the plants and extracting some of the coloring matter with 95 percent alcohol. The resistance to sunlight of the chlorophyl thus recovered from the sprayed plants seemed to be somewhat greater than that derived from the unsprayed plant, but the differences were not marked. The difficulty experienced in extracting the chlorophyl by means of alcohol from sprayed plants already has been noted by Ewert (16). It is a slow process even after the plants have been boiled previously in water. The copper salts evidently enter into some sort of chemical union with the chlorophyl, forming a substance which is very tenacious in its hold on the proteid substratum of the chlorophyl bodies and which renders the coloring matter much more stable in the presence of direct sunlight. The determination of the nature of this combination is a difficult chemical problem, but the compound called by Tschirch (43) copper phyllocyanate probably is the one which is formed. Tschirch found that the chlorophyl in the skins of the grapes unites during the process of fermentation with the copper salts derived from this compound in an insoluble form, thus serving to remove the copper from the wines made from grapes that have been sprayed.

(c) WHAT EFFECT HAS COPPER ABSORPTION ON LEAF FUNCTIONS?

An increase in the amount of assimilated carbon dioxid apparently is the visible external evidence of the absorption of copper by the potato leaf. Under American climatic conditions, sprayed plants always yield more tubers by weight than do unsprayed ones. The external appearance of the sprayed plants—dark green foliage and thick stalks—apparently would confirm this result. On the other hand, the early death of the unsprayed rows would seem to indicate that this increase is due in the main to the longer life of the unsprayed plants. The problem is further complicated in America by the presence of tip-burn. The cause of this disease has been discussed at much length in former publications of this Station and is further reviewed on page 79 of this bulletin. It always is present in this vicinity during August and may appear as early as July 15. Its effects are shown much more severely on the unsprayed plants than on those treated with bordeaux. An attempt has been made during two years to determine whether spraying increases the yield even before tip-burn gets a hold on the plants and kills much of the foliage. This attempt was more easily stated than accomplished for the reason that tip-burn is liable to show itself before much spraying can be done, and before its effect becomes manifest on the foliage. The earliest harvest of potatoes for this purpose was made in early September, and even at this date tip-burn already had done some foliage damage, as may be seen by an examination of the comparative weights of the tops of the sprayed and unsprayed plants.

	Large tubers	Small tubers	Total	Average weight of tops
	bushels	bushels	bushels	pounds
1910 Bordeaux	167.9	26.9	194.8	0.809
Sept. 2 Control	130.5	39.2	170.	0.753
1912 Bordeaux	104.5	15.3	119.8	0.668
Sept. 4-5 Control	73.1	14.	87.	0.475

These results show, however, that copper mixtures exert a favorable effect on the plant within a short time after they are applied. The tip-burn and flea beetle already have done more injury to the control plants within this short time than to the sprayed ones and it is doubtful if field experiments can be carried out in Vermont which will be free from this difficulty. Furthermore, the inevitable and unavoidable differences in soil in several parts of a field also lead to yield variations that are likely to convey false impressions. Even when an experiment is repeated a number of times in the same field, the effects of this

source of error are not nullified. It is only when results are obtained for a series of years and from a number of plots and when the differences are pronounced that one can dogmatize as to the outcome of field experimentation. Hall and Russell (19), as a result of the three-quarters of a century of soil and plot work at Rothampsted, where the operations always have been done with the utmost care and where the general conditions tend to favor accuracy in experimental work, consider it unsafe to lay stress on differences of less than 15 percent. Experiments made under greenhouse conditions and pot experiments conducted after the manner of Ewert's trials alone will serve to enable one definitely to determine whether spraying a plant actually causes its stimulation and an increase in its daily starch output. However, greenhouse experiments do not duplicate our weather conditions either as to light, temperature or humidity, and are of interest only when viewed from a scientific standpoint and for the particular conditions which they represent. It would seem, therefore, that pot experiments, so conducted as to give to all the plants uniform soil conditions and as to control the open air surroundings, temperature, light and humidity, represent the only safe procedure to adopt.

Granting that some of the physiological changes are brought about in the plant and are expressed in their varied yields, it is next necessary to inquire into the ordinary functions of the plant in order to determine, if possible, the one affected by the presence of the spray mixture on the outside of the foliage and of traces of copper salts inside the plant. These functions may be summarized as:—

The absorption of water by the roots.
The transpiration of this water by the leaves.
The assimilation of carbon dioxid for starch formation.
The removal of the products of starch assimilation.
The respiration of carbon dioxid.
The formation of proteids and other nitrogenous products.
The synthesis and destruction of chlorophyl.

TRANSPIRATION

The absorption of water by the roots and its transpiration by the leaves are correlated so nearly that they may be considered together. A comparison of sprayed and unsprayed plants would lead inevitably to the conclusion that the former took up more and gave off less water than the latter. Their succulent foliage and comparative freedom from tip-burn tells the story. Tip-burn has all the superficial appearances of a disease caused by a lack of water or by excessive transpiration.

Data on the effect of bordeaux on transpiration are very contradictory. Rumm (35) and Schander (38) are of the opinion that it tends to lessen the amount of water given off, and Ewert (16) is apparently one with them in their belief. On the other hand, Frank and Krüger (17) Zucker (47) and, more recently, Duggar and Cooley (13, 14) have shown that in potted potato plants the leaves covered with bordeaux mixture transpire more water than do those which lack such a covering. This difference was increased further by the addition of lampblack to the bordeaux and was true also of leaves covered with the lime wash. The lime-sulphur probably was too dilute (1 to 25) to produce very much effect. It would seem as if a problem of this nature would be solved easily. There must be some flaw in the methods used or the results obtained would have been more consistent.

In the first trials conducted at this Station, branches of bordeauxed and unbordeauxed plants were brought in from the field and the amount of water transpired in the shade for a period of about two hours determined by means of a Ganong potometer. The leaves were weighed in one trial, and in the others the leaf surfaces were determined by means of a planimeter.

No.	Plant	Time[1]	Total water transpired cc.	Transpiration per minute per gram of leaf weight cc.	Leaf area square inches	Transpiration per minute per square inch leaf surface cc.
1.	Unbordeauxed,	9:49-11:48 a. m.	.6 in 119 min.	.00059		
2.	Bordeauxed,	9:40-11:54 a. m.	.75 in 137 min.	.00076		
3.	Unbordeauxed,	4:00- 6:15 p. m.	1.26 in 124 min.		37.50	.000266
4.	Bordeauxed,	4:00- 6:35 p. m.	.92 in 146 min.		47.82	.000131
5.	Unbordeauxed,	2:38- 4:21 p. m.	1.54 in 97 min.		56.26	.000187
6.	Bordeauxed,	2:38- 4:45 p. m.	1.18 in 120 min.		52.49	.000282
7.	Unbordeauxed,	8:48-11:05 a. m.	1.00 in 134 min.		51.39	.000145
8.	Bordeauxed,	8:48-11:32 p. m.	.92 in 157 min.		50.44	.000116
9.	Unbordeauxed,	8:54-11:16 p. m.	1.77 in 115 min.		53.89	.000285
10.	Bordeauxed,	8:57-11:54 p. m.	1.20 in 177 min.		43.04	.000157

Average of four unbordeauxed plants per square inch of leaf surface per minute ....000229 cc.

Average of four bordeauxed plants per square inch of leaf surface per minute ....000171 cc.

These results were obtained on five different days. They seem to show that under these conditions the unbordeauxed plants transpire more water than the bordeauxed ones. The brief time during which the leaves were under observation as well as the unusual conditions under which the plants were placed, led the writer to feel that perhaps

[1]The time is given only to indicate the part of the day in which the experiment was performed. In a majority of the experiments some time was taken out while the apparatus was being readjusted.

they were not transpiring at all normally. The amount of water transpired per minute per square inch of leaf surface was so very small as to raise doubts in his mind. Hence other means were devised in order to check these results. The simplest of these was aimed to determine the amount of water given off by the treated and untreated potato plants when wilting. Three large potato branches that had been bordeauxed and three slightly smaller ones that had not been thus treated were brought from the field into the laboratory at nine o'clock. They were placed on a table, in the shade, near a window through which a current of air was passing. Their weights were:—

	9 A. M. grams	2:30 P. M. grams	Loss grams
Bordeauxed plants	174	130.5	43.5
Unbordeauxed plants	132	113.	19.

A similar experiment on the following day, using eight branches resulted as follows:—

	9:20 A. M. grams	2:30 P. M. grams	Loss grams
Bordeauxed plants	219	172	45
Unbordeauxed plants	214	184	30

These eight branches were kept in the laboratory for a week and then were placed in the open sunshine for three days to air dry.

	Weight after a week had elapsed grams	Weight after three days more additional sunshine grams	Loss from original weight grams	Percentage loss
Bordeauxed plants	35	18	201	91.8
Unbordeauxed plants	46	23	191	89.2

This result also would seem to indicate that the bordeauxed plants were slightly more succulent than the unbordeauxed ones.

Similar trials were made with sprayed and unsprayed potato (Industrie) plants from the Economic Botanical Garden at Bonn. The weights of the young and old leaves were kept separate.

	Weight at 10 A. M. grams	Weight at 12 A. M. grams	Loss grams	Percentage loss
6 old leaves, bordeauxed.........	25.5	19.5	6.	23.5
25 young leaves, bordeauxed....	22.8	17.9	4.9	21.
6 old leaves, unbordeauxed.......	29.3	25.	4.3	15.
25 young leaves, unbordeauxed...	19.	17.3	1.7	9.

Leaves taken from freshly bordeauxed plants gave these results:—

	Weight at 8:30 P. M. grams	Weight at 2:30 P. M. grams	Loss grams	Percentage loss
7 old leaves, unbordeauxed......	35.5	30.3	5.2	14.5
30 young leaves, unbordeauxed..	20.	18.7	2.1	9.
12 old leaves, bordeauxed........	41.5	34.4	7.1	17.
30 young leaves, bordeauxed	18.7	16.6	2.1	11.

On August 9 several plants were allowed to air-dry:—

	Weight at 10:30 A. M. August 9 grams	Weight August 17 grams	Loss grams	Approximate percentage dry matter
11 large leaves, bordeauxed......	35.3	7.3	28.	20.
32 small leaves, bordeauxed	22.3	4.2	18.1	18.8
9 large leaves, unbordeauxed....	43.7	7.4	36.3	16.9
34 small leaves, unbordeauxed....	25.7	4.8	20.9	18.5

The bordeaux still clinging to the sprayed plants readily would account in this case for a part of this apparent difference in the percentage of dry weight. The plants had been sprayed recently and there was considerable copper salt still adhering to the dry foliage.

FIG. 10. Apparatus used in transpiration experiments.

The study of the transpiration end of the problem was resumed in the spring of 1914, using potato plants grown in the greenhouse. These plants were sprayed twice in order to insure a thorough coating of the leaves with the mixture, and being about two-thirds grown, were placed in potometers of the form shown in figure 10. Plants as nearly as possible of the same size and shape as the sprayed ones were placed in other potometers. It was found easier to ascertain moisture losses by weight than by measurement. Three trials were

carried on inside the greenhouse during March and April, and three were conducted for the most part in the open air in early September, using plants grown and sprayed in the open. The plants were weighed twice daily and, after the conclusion of the experiment, the leaves were removed and weighed. Leaf surface areas were determined by means of a planimeter during the first two experiments.

	Weight of leaves grams	Leaf surface sq. cm.	Total transpiration grams	Transpiration per gram of leaf grams	Transpiration per sq. cm. grams	Transpiration per sq. cm. grams
			GREENHOUSE TRIALS			
Unbordeauxed ..	27.5	103.9	121.5	4.4	1.17	
Unbordeauxed ..	55.5	274.9	194.	3.5	0.70	0.93
Bordeauxed	25.5	96.27	130.5	5.1	1.35	
Bordeauxed	43.5	181.15	186.	4.3	1.02	1.18
Unbordeauxed ..	42.5	169.73	133.	3.1	0.78	
Unbordeauxed ..	42.5	194.81	145.	3.4	0.74	0.76
Bordeauxed	38.5	164.9	147.	3.8	0.89	
Bordeauxed	30.5	125.35	157.	5.1	1.25	1.07
Unbordeauxed ..	18.		73.	4.		
Unbordeauxed ..	23.		67.	2.9		3.5*
Bordeauxed ...	31.		111.	3.6		
Bordeauxed ...	30.		102.	3.5		3.6*
			OUT-OF-DOOR TRIALS			
Unbordeauxed ..	92.5		361.	3.9		
Unbordeauxed ..	87.		295.	3.4		3.7*
Bordeauxed ...	132.		421.	3.1		
Bordeauxed ...	132.		526.	3.9		3.5*
Unbordeauxed ..	130.		933.	7.1		
Unbordeauxed ..	169.		830.	4.9		6.*
Bordeauxed	124.		977.	7.9		
Bordeauxed ...	169.		906.	5.3		6.6*
Unbordeauxed ..	192.5		468.	2.4		
Unbordeauxed ..	186.5		365.	1.9		2.2*
Bordeauxed ...	234.		515.	2.2		
Bordeauxed ...	189.		414.	2.2		2.2*

An examination of these data shows that in two cases more water was given off in the greenhouse by the bordeauxed plants than from the unsprayed ones and that in the third the results were indeterminate. In one of the experiments made in the open air water losses from sprayed plants exceeded those from unsprayed plants, and in the other two trials the results were essentially alike.

The results of these experiments may be summed up as follows:

		Greenhouse	Open air
Weight of leaves (grams)	Unbordeauxed	209.0	857.5
	Bordeauxed	199.0	980.0
Transpiration (cubic centimeters)	Unbordeauxed	733.5	3252.0
	Bordeauxed	833.5	3759.0
Transpiration (cubic centimeters per gram weight)	Unbordeauxed	3.5	3.79
	Bordeauxed	4.2	3.83

*Average transpiration per gram of leaf stated as grams.

The general conclusion that may be drawn from the foregoing tabular statement is that transpiration is slightly increased by spraying, especially under conditions where there is no rapid circulation of air. The difference is so slight, however (only about 1 percent) that the small loss readily might be compensated for by the plant. Even the interjection of a pane of glass between the plant and the sunlight is sufficient to change the amount of light and heat. The only thing than can be said of such experiments is that while they are correct for the given conditions they might not hold for others.

RESPIRATION

No experiments have been conducted at this Station to determine the effect, if any, of spraying on the passage of carbon dioxid from the plant. . Any attempt carefully to control an experiment leads to the introduction of all sorts of abnormalities, and no method has been devised as yet that will avoid such difficulties. Ewert's (16) careful experiments have shown that a bordeauxed plant requires much less carbon dioxid than does an unbordeauxed one when the two are placed under a belljar and the amounts of respired carbon dioxid are measured. The condition and behavior of a plant growing under a belljar must be different from that of the same plant in an open field.

STARCH REMOVAL

Sections of leaves that have been sprayed with bordeaux usually show, as was figured by Frank and Krüger (17), chlorophyl bodies stuffed with large starch grains while the same organs in the unsprayed leaves contain fewer and smaller grains. Ewert (16), while not denying the presence of an increased starch content in the sprayed leaves, asserts that its presence is due to the inability of these cells to remove it as fast as it is formed. He has shown that copper compounds retard the action of diastase and that an accumulation of starch occurs in the chlorophyl. Sprayed leaves, decolorized by the action of alcohol with their starch stained blue with iodin according to Sach's method, show bluish patches even after they have been kept in darkness for 24 hours, whereas untreated leaves kept under similar conditions lose all their starch. It is comparatively easy to confirm the observations both of Frank and Krüger and of Ewert. The starch in bordeauxed leaves, even after they have been kept in darkness for some time, still remains in spots. These starch-holding areas seem to exhibit no regular arrangement or size, but they occur more frequently near the base of the leaves than elsewhere. An attempt was made to

correlate them with injured portions of the leaf, but, so far as could be made out, the parts of the leaf containing starch were as normal as the remainder. The probability is that these parts were covered by a layer of bordeaux in which much free copper had been given off and under which the cells had absorbed enough to prevent the action of the diastase. The action of the copper salts is local in its effects. Spots of this nature would be likely to result if any appreciable amount of copper were absorbed by the underlying tissues.

CARBON DIOXID USAGE

Does a sprayed plant use more carbon dioxid than an unsprayed one? In studying this question Amos (2) used the Brown and Escombe method. Similar leaves from the same plant were taken, air and carbon dioxid drawn through glazed cases in which the leaves are exposed and the carbon dioxid contents of the inspired and expired air were determined. The results obtained on grape leaves are fairly typical. The ratio expresses the average absorption per square decimeter, the sprayed leaves being represented as unity.

July 28, 29, 31	1 : 1.02
August 4, 5, 10	1 : 0.79
August 18, 20	1 : 1.03

Amos inferred that the application of bordeaux mixture to plant leaves diminishes their carbon dioxid assimilation for a time, but that this effect gradually wears off.

(d) Shading and Temperature Effects

The intensity of the sun's rays is much reduced to a leaf which is covered by a heavy coating of bordeaux mixture. This effect is of much more importance in America than in the great European potato growing countries for the reason that, owing to our lower latitude, the light is much more intense. The air, too, is less hazy than in England and Germany where the moisture laden west winds blow.

The bordeaux-mixture collects and dries on the tips or along the veins of a leaf. These portions are thus protected from the destructive action of the sunlight on the chlorophyl. The sunlight probably exerts both light and heat effects on the leaf. The amount of protection afforded by the bordeaux of course is entirely dependent on the thickness of the layer on the leaf, and this varies on different leaves and on different portions of the same leaf. A moderately thin layer will reduce the light intensity by one-half while a very thick one will allow only the passage of about a fourth of the light. Schander (38) has

emphasized, perhaps too strongly, this effect of bordeaux in Germany where light intensities are relatively weak.

Leaves protected by bordeaux are slightly cooler than those which lack this covering. A thermometer held under a glass plate covered with a thin layer of the 5-5-50 mixture upon which direct sunlight pours, registers from a third to two-thirds of a degree Centigrade less temperature than when exposed to the direct sunshine. This difference, while slight, ought to be sufficient to reduce the evaporation of the leaf and to protect to some extent the chlorophyl in the palisade parenchyma from excessive destruction. Chlorophyl seems to be much more easily broken down in the potato leaf than in most plants. Incipient tip-burn usually is indicated by a yellow color of the leaf which spells chlorophyl destruction.

VIII. FLEA BEETLE INJURIES

The study of the problems of the physiology of sprayed and unsprayed potato plants in the field is complicated further in America by the holes made by the flea beetle. These injuries are much more serious on unsprayed than on sprayed plants, the number of holes being from three to four times as great. An examination of several medium sized unsprayed leaves made during the summer of 1914 showed punctures ranging in number from 30 to 112. These holes are about 0.5 to 1 millimeter in diameter. From two to six percent of the leaf structure is thus lost, whereas not more than one or two percent is eaten in the sprayed leaves. The flea beetle undoubtedly

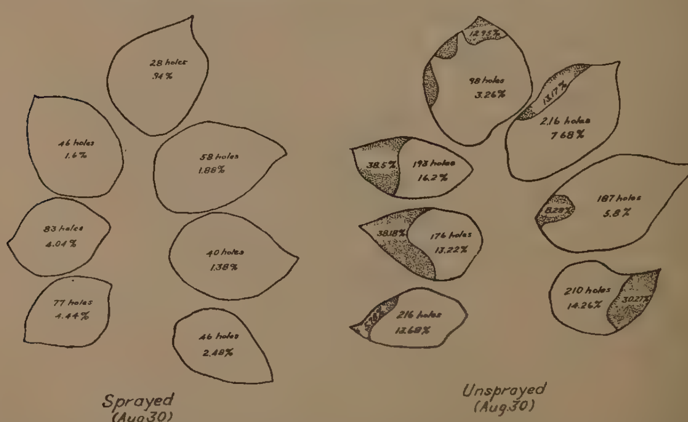


FIG. 11. Relative loss from flea beetle and tip-burn injury on sprayed and unsprayed leaves of Irish Cobbler potatoes.

on the average is responsible for about one-fifth of the loss in yield in unsprayed plants; or, reversing this statement and phrasing it in a positive manner, it is not too much to say that under normal and average seasonal conditions in this vicinity in years when true blights are absent, not far from a quarter part of the gain secured by the use of bordeaux on potatoes may be attributed to its deterrent effect as against the flea beetle; in some years more, in other years less. The flea beetle seems to be most active during those seasons when fungus diseases are not prevalent, and the plants usually are considered to be normal and healthy. Under such conditions spraying usually is worth doing, even though it only wards off the worst of the insect attacks.

In this connection the following statement as to the flea beetle injury studies is of interest. Equal numbers of thoroughly representative Irish Cobbler leaves from all parts of the plants both sprayed and unsprayed were copied in outline on August 30, 1915. The sprayed plants showed little or no tip-burn injury whereas the unsprayed ones already had suffered considerably. The flea beetle punctures for each leaflet were counted and noted and the amount of tip-burn injury was indicated as nearly as possible. Total leaf areas and also total tip-burn areas were then measured by means of a planimeter.

Area of sprayed leaves	1,074.6 sq. cm.
Area of unsprayed leaves	936.8 sq. cm.
Area of tip-burn on sprayed leaves	none
Area of tip-burn on unsprayed leaves	201.0 sq. cm.
Number of holes in sprayed leaves	2,465. sq. cm.
Number of holes in unsprayed leaves	7,901. sq. cm.
Assuming 150 flea beetle punctures to each centimeter.	
Area lost by flea beetle injury, sprayed	16.4 sq. cm.
Area lost by flea beetle injury, unsprayed	52.6 sq. cm.

Of course the flea beetle punctures could be counted only on the portions of the leaf that were still intact. Assuming that there was the same proportion of injury on the tip-burn portion, the total leaf area removed by the flea beetle would approximate 65 square centimeters.[1] The percentage then would be about as follows:—

Area of unsprayed plants lost by tip-burn	21.4 percent
Area of unsprayed plants lost by flea beetle	6.9 percent
Area of sprayed plants lost by flea beetle	1.5 percent

IX. GENERAL CONCLUSIONS

The physiological effects of bordeaux mixture on potato plants in the open field in this climate seem to be favorable. The writer ascribes

[1]In this connection should be recalled the flea beetle counts on sprayed and unsprayed leaves made twenty years ago at this Station by Jones (22), who found even a smaller number of punctures on the sprayed plants than are reported here.

this result entirely to lessened tip-burn and flea beetle injuries. His earlier opinion, as expressed in bulletin 159, to the effect that spraying plants seems to tend to stimulate and to augment the daily production of starch, seems to be incorrect. The life of the starch making organs is prolonged, but these organs are not stimulated by the copper salts daily to produce more starch. The application of the copper-lime compounds is to be looked on as a protection rather than as a stimulus. Under indoor conditions there probably results from spraying a general lowering of all functions of the plant unless it be that of transpiration. Under field conditions transpiration seems to be greater from the sprayed than from the unsprayed plants, but the lowering of the other functions impairs the vigor of starch formation and removal, so that no real increase occurs from day to day. It must be admitted that the influence, helpful or harmful, of bordeaux spraying on plant life has been much overestimated, as Chuard and Porchet (8) already have pointed out on the grape. The blue-green color imparted to the foliage of sprayed plants is likely to lead the observer in the field to the conclusion that the plants are healthier than normal and that their yields will be greater. The results secured from spraying are very favorable, but the apparent cause already has been stated.

It is probable that a very even balance is maintained between favorable or unfavorable effects from spraying prior to introduction of the tip-burn. The bordeauxed plants then have the advantage. This complication is introduced so early in the problem that it is impossible to decide from early harvests, as was tried in 1911 and 1912, whether the mixture applied early in the season, had any effect on the plants. The loss in yield on the unsprayed plants as early as September 1, due to tip-burn and flea beetle injuries, is sufficient to vitiate any comparisons. The loss of foliage from tip-burn occurs earlier than the writer noted in 1911, as is shown by the fact that the unsprayed plants were somewhat lighter.

The recourse to greenhouse experimentation and to the spraying experiments in Germany was had with the intention of eliminating these factors. The numerous conflicting German tests (24) have been made where tip-burn does not occur. It is practically unknown there, although the writer has been informed by competent authority that it did occur in Germany to a slight extent during the very hot, dry summer of 1911. Tip-burn and flea beetle being eliminated, no favorable results were secured from spraying in the experiments at Bonn or in the greenhouse in 1914. The potatoes in the greenhouse in 1915 were

benefited by spraying, but for some reason tip-burn was almost as severe that summer indoors as it was outdoors.

It must be further pointed out that the results secured in spraying of beans and artichokes confirm the work at Bonn and in the greenhouse on potatoes. These two plants are not subject to tip-burn. They offer an uncomplicated opportunity to measure the stimulating effect of bordeaux, if any such exists. In no case did the use of bordeaux show the slightest result. The objection may be raised that these plants have thicker cuticles and that consequently they do not absorb the poison as readily as does the more tender leaf of the potato. This may be true, since neither the bean nor the artichoke is as succulent as the potato; or they may not be as readily affected, at least by the small quantities they cannot help absorbing. Bayer (6) noted decided changes in the appearance of sprayed bean foliage, but the writer has seen no such dark green color on either beans or artichokes.

The relative importance of tip-burn and flea beetle injury already has been discussed. The experience of two years based on estimates and actual counts of holes and of size of tip-burned areas, has led to the conclusion that the flea beetle causes from 10 to 30 percent of the total leaf losses, while tip-burn is responsible for from 70 to 90 percent. These proportions naturally vary from year to year and probably do not hold for locations other than Burlington.

Frank and Krüger's (17) contention that the increased yield secured in their experiments as a result of using bordeaux was due to the more rapid transpiration of the sprayed plants, in part is confirmed by the present work. An increase in transpiration connotes an increase in the green weight of a plant, as has been shown by Livingston (27); in fact, the transpiration of the plant is a fairly accurate measure of its size. Against this idea, that increased transpiration is an advantage, must be balanced the fact that the potato often must find it difficult to maintain the water content of its leaves. On the other hand, however, it must be recalled that the potato very rarely is found wilting in the field.

The increase in transpiration brought about by the use of bordeaux probably is a purely physical phenomenon. The use of other powders and spray mixtures doubtless would cause the same results and affect the plant in the same way, so far as water losses are concerned. The dried films or powders pressed close to the cuticle draw water from the epidermal cells and from the deeper lying palisade parenchyma. However, our 1909 spraying trials showed that lime did

not increase yields and the New York (Geneva) Station has found that lime-sulphur at times is even injurious to the plant. Potato roots in certain dry seasons and in dry, sandy soils at times must be put to it to supply the plant with the necessary moisture. It may be, too, that in a very damp climate with a minimum of sunshine bordeaux would exert the opposite effect on the plant, that is to say that it would cause a lessened transpiration. However, our greenhouse experiments would indicate otherwise. The stomata to some extent must be plugged by the bordeaux, but not to an extent sufficient to overcome the more rapid transpiration from the leaf surface induced by its use.

The most interesting problem in this entire study from the standpoint of pathology is the determination of the manner in which the copper salts prevent tip-burn. No reasonable doubt remains that these are absorbed in small quantities by the leaf. Dandeno (10) found that the copper from a dilute solution of one of its salts, allowed to dry on a primula leaf, left no stain. Chemical tests of the leaves show that the copper enters in noticeable quantities, and the diastase reaction of Ewert (16) is still more conclusive. A chemical combination is formed with some of the cell constituents, probably the chlorophyl. The difficulty with which the chlorophyl is removed from sprayed leaves indicates that it has been so changed that it is much less soluble than heretofore. Our knowledge of the synthesis and destruction of the chlorophyl is even more vague than that of the part it plays in starch formation. Anything that could be offered would be in the nature of a surmise. A comparison of this chemical substance with others that occur in the cell would lead to the conclusion that like them it is consumed as an ultimate result of its life processes. Therefore it must be continually renewed. It would seem that American sunlight is so brilliant that the chlorophyl of the potato plant is very easily destroyed. It may be that only certain portions of the molecule break down under its influence. Tip-burn usually shows first as a yellowing of a portion of the leaflet; and from this fact one would surmise that it is the constituent that gives the chlorophyl its blue or blue-green color that is lost earliest. It would appear that the copper sulphate unites with this portion and makes it more stable and more capable of maintaining its structure even in the face of brilliant, intense sunlight. The chlorophyl molecule, if it is one substance, seems to be a structure the whole edifice of which collapses if a single one of the important stones crumbles. Tip-burn, except in its last stages, is an injury due to the lack of water rather than to a pathological condition.

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